Studies on selenium in plants and soils. (Thesis)

Bisbjerg, B.

Publication date:
1972

Document Version
Publisher's PDF, also known as Version of record

Risø Report No. 200

Danish Atomic Energy Commission

Research Establishment Risø

Studies on Selenium in Plants and Soils

by Birte Bisbjerg

January 1972

Sales distributors: Jul. Gjellerup, 87, Sølvgade, DK-1307 Copenhagen K, Denmark
Available on exchange from: Library, Danish Atomic Energy Commission
Risø, DK-4000 Roskilde, Denmark

UDC 631.4:633:636

STUDIES ON SELENIUM IN PLANTS AND SOILS

by

Birte Bisbjerg

January 1972 Risø Report No. 200

STUDIES ON SELENIUM IN PLANTS AND SOILS

by
Birte Bisbjerg

Danish Atomic Energy Commission
Research Establishment Risø
Roskilde

Denne afhandling er af Den kgl. Veterinær- og Landbohøjskoles fagråd for landbrugsvidenskab antaget til offentligt at forsvares for den jordbrugsvidenskabelige doktorgrad.

København, den 10. maj 1973.

J. Wismer-Pedersen
Formand for fagrådet for landbrugsvidenskab.

ISBN 87 550 0118 1

CONTENTS

INTRODUCTION

For many years the lack of suitable analytical methods forced the investigators of the element selenium to deal exclusively with the cases where its presence in excess amounts posed a toxicity problem. The advent of highly sensitive analytical tools, first and foremost the radiometric ones, has in the last ten to twenty years enabled experimenters to approach the questions concerned with selenium deficiency. For the present work the research reactors and facilities for radioactivity work at Risø have been used in a study of the role of selenium in Danish agriculture.

During the past decade increasing attention has been paid to the importance of selenium to livestock, and deficiency has, indeed, been found in many areas all over the world. It was therefore felt that an investigation of the selenium content in Danish crops was very important. The frequent observation in recent years of Danish domestic animals affected with diseases that might have been caused by selenium deficiency was an indication that such a study might be rewarding.

If the selenium concentration in the crops is below the desired level, a possible remedy is the use of fertilizers containing selenium. A prerequisite for such an application is the knowledge of the quotient of absorption which among other things depends on the crop, on the selenium compound and on the soil properties. Other important factors are changes in availability with time, the extent to which leaching of the added selenium takes place, and the probability of accumulation in the soil. All such questions, therefore, have to be considered. Results from other countries can be indicative, but they are not always immediately applicable to Danish conditions.

In outline the contents of the present report are as follows. The first chapter is a review of the literature of greatest interest for this treatise, but it is intended also as a general introduction to the subject. The description and evaluation of the experimental techniques are given in chapter 2. The work depends heavily upon the use of radiochemical methods. Owing to the volatility of some selenium compounds an additional problem was the necessity of controlling of the reliability of all steps in the process. In chapter 3 the analytical results are presented for crop and soil samples from various districts in Denmark. Besides, results are presented of experiments concerning the absorption by plants of native selenium. Chapter 4 comprises a series of experiments where the selenium concentration in plants was increased as a result of the adding of selenium compounds to the soil. In chapter 5 the conversions of selenium compounds added to soil are studied by means of extraction procedures. Chapter 6 concerns the influence of selenium upon the sulphur concentration in plants. A general conclusion based upon the results is given in the last chapter. In the Appendix some chemical properties of selenium are given that are considered of interest for the present investigation.

1. SELENIUM IN SOILS, PLANTS AND ANIMAL NUTRITION

In addition to an ever increasing number of publications in periodicals, two books deal with the biological aspects of selenium. One is "Selenium, Geobotany, Biochemistry, Toxicity and Nutrition" by Rosenfeld and Beath (Ros 1964)*, a thoroughly revised edition of "Selenium" by Trelease and Beath (Tre 1949). The other is the proceedings of a symposium with the title "Selenium in Biomedicine" (Mut 1967).

A comprehensive review of the literature should therefore not be necessary. Instead, the present chapter contains a selective and up-to-date review of the part of the literature that is especially relevant to selenium in soils and plants. Hopefully, this chapter may serve as an introduction to the subject, which is new to Danish agriculture. In the interest of brevity results to be discussed in the following chapters are not included in the review, except in form of a reference to the chapter in question.

1.1. Soil Selenium

The concentration of selenium in a soil depends primarily upon the parent rocks, the way the land was formed, the climate, the topography, and the age of the material. The chemical forms of selenium in a soil are a result of the properties of the soil, in particular the pH, the oxidation potential and the presence of iron.

1.1.1. Geochemistry

In unweathered rocks and mineral ores the occurrence of selenium is nearly always connected with sulphur minerals. The radii of S^{--} and Se^{--} are 1.74 and 1.91 Å respectively, and thus selenium can replace sulphur in sulphides. The extent of replacement is larger at high than at low temperatures. In addition to the sulphides volcanic sulphur may have a considerable selenium content.

The abundance in the lithosphere of selenium relative to sulphur is on the average 1:6000. Further, selenium appears to be approximately fifty times as abundant as tellurium, the next element in the oxygen group. The absolute sulphur content is not precisely known because the mass of the sulphide ores is uncertain, but based on the igneous rocks the average sulphur concentration is 520 ppm. Thus a selenium concentration of 0.09 ppm (Gol 1954) or 0.05 ppm Se (Tur 1961) is obtained.

* References are given by the first three or four letters of the name of the first author followed by the year of publication. The reference list pertaining to all chapters is at the end of the book.

During weathering the behaviour of sulphur and selenium differs. The main reason is that while sulphide is readily oxidized into sulphate, the oxidation potential will mostly not be high enough for a similar oxidation of selenide, and the process stops with the formation of selenite. Only when the oxidation potential is very high, will the oxidation go on to Se^{+6}. Nitrate deposits are the extreme examples of this.

The cycle of sulphur is roughly as follows. The sulphate formed in the oxidation zone may enter a cycle between plants, animals and micro-organisms, but under a humid climate a large part of it will leach out and be taken to the ocean with the rivers. The sulphate not remaining in the sea water is distributed on the sediments either as sulphates of the alkaline earth metals, as heavy metal sulphides or as elemental sulphur. Further the sulphate may be found in evaporates and sometimes in precipitates. Only little sulphur remains in the resistates, e.g. sandstones.

Selenites brought into solution will readily be removed again, and only very little is refound in the sea water. The selenites may be absorbed and coprecipitated with the iron and manganese hydroxide sediments and with organic material. Therefore shales rich in iron, manganese or carbon often have a large selenium content. Further the selenites may be reduced to the elemental form or to selenide. Mostly sandstones contain little selenium, limestones slightly more, but still less than shales. Some figures are given in table 1.1 to illustrate the variation in the concentrations. The selenium concentration in sea water is relatively low as compared with the sulphur concentration, and hence the selenium content is very low in many evaporates. Only in such evaporates as nitrate deposits has the selenium - here oxidised to selenate - followed the sulphate.

The difference in the cycles of sulphur and selenium is well illustrated by the geochemical balances of the two elements. On the basis of the amount of sodium present in sea water and sediments today Goldschmidt has estimated

Table 1.1

Average selenium and sulphur contents of rocks and sediments (Tur 1961)

	ppm Se	ppm S	S: Se
Igneous rocks	0.05*)	300	6,000
Shales	0.6	2400	4,000
Sandstones	0.05	240	4,800
Carbonates	0.08	1200	15,000
Deep-sea sediments clay and carbonate	0.17	1300	7,700

*) Selenium calculated from an assumed S : Se ratio of 6000 : 1.

Table 1.2

Geochemical balance of sulphur and selenium. The balance is based upon data in refs. Ran 1950, Gol 1954 and Schu 1965.

	Sulphur g/cm^2 *)	Selenium g/cm^2 *)	S : Se
Released from igneous rocks	80	0.014	6,000
Present in sea water	245	0.00003	8,000,000
Present in sediments	310	0.1	3,000

*) grams pr cm^2 of the earth's surface

the amounts of weathered igneous rocks and of sediments formed to be 160 and 155 kg pr cm^2 of the earth's surface respectively (Gol 1954). With the established average concentrations in the two materials and in sea water, the amount of which is 280 kg per cm^2, the figures in table 1.2 are obtained. The figures illustrate two points. Firstly, the difference in behaviour of sulphur and selenium as seen from the large S:Se ratio in sea water. Secondly, it is seen that the amounts of the two elements present in sediments plus sea water by far exceed those released during weathering. This excess must derive from volcanic emanations.

1.1.2. Selenium Concentration in Soils

For a discussion of the selenium concentration in Danish soils the reader is referred to chapter 3.2. Globally most soils contain 0.1 to 2 ppm Se (Swa 1955), of which only a small part is in general available to the plants. Vinogradov gives the value 0.01 ppm for the total content, but this is obviously too low (A. P. Vinogradov 1959, cited in Lak 1967). In some cases, however, the amount of available selenium is of such a magnitude that the plants become toxic to animals. It is common practice to call a soil seleniferous when the total content of selenium is appreciably above normal, and the term will be used in the present work when the total content is 2 ppm or more. The term toxic is used when the crops produced on a soil are toxic to animals.

Many soils have been analysed for their total content of selenium, but only in a few cases have the crops grown on the soils been analysed too. The data in table 1.3 are primarily for soils where both the soil and the plants have been analysed. Except for New Zealand the data are not representative for the country either owing to a small number of samples or owing to a specific purpose of the sampling. The Hawaiian soils are an example of seleniferous, but non-toxic soils.

Table 1.3

Selenium content of soils and plants

Country	No. of soil samples	ppm Se in soils		ppm Se in plants	Ref.
		Range	Ave.		
New Zealand	27	0.1 – 2		<0.01 – 0.05	Wat 1962
New Zealand	62	0.12 – 3.5	0.60		Wel 1967
USA	8	0.06 – 0.62	0.3	low	Cary 1967
Denmark	7	0.16 – 1.5	0.4	0.03 – 0.5*)	Gre 1964
Hawaii		0.4 – 26		< 3	Bye 1938
Western USA	500	<1 – 80	4.5	>50	Tre 1945
Ireland	19	1.2 – 324	30	0.8 – 450	Fle 1957, 1962a
Sweden	24	0.16 – 0.98	0.39	0.006 – 0.064	Lin 1970

*) Pot culture

Soils producing toxic crops are found in all continents. The most well-known areas are in North and South Dakota, Montana, Wyoming, and Colorado in the U.S.A., and in Canada north of Montana and N. Dakota. Further, toxic areas have been found in South America, South Africa, Israel, Queensland (Australia), and in Ireland. The selenium in most of these areas derives from seleniferous sediments, most often shales, from which selenium is made soluble during weathering. Sulphidic ores with a considerable selenium content are common in many of these areas. The selenium content in sulphidic ores varies much. Thus a series of American pyrites contained from trace to 400 ppm Se (ave. 70 ppm), but in other samples as much as 13 % Se has been found (Bye 1935). Furthermore volcanic emanation during earlier geologic periods is supposed to have contributed to for instance the toxic areas in North America.

Generally the toxic soils are neutral or alkaline, and the precipitation is moderate. The soluble selenium has either remained in the soil or has been transported to lower-lying areas where the concentration is increased. A special example is some toxic soils in Ireland, where selenium has accumulated in poorly drained valleys rich in organic material (Fle 1957). Thus the highest concentration measured in a soil - 1200 ppm Se - has been found in a highly organic horizon of an Irish soil.

The other extreme is the soils giving crops with such a low selenium concentration that deficiency symptoms develop in the livestock. The areas where selenium deficiency occurs have some characteristics in common. The sediments contain relatively small amounts of selenium, and the soils are not fed with selenium from the weathering of neighbouring areas. The amount of precipitation is mostly moderate to high, and leaching plays an important role. The role of the annual precipitation has been illustrated in a single

investigation showing that in pastures in Western Australia the selenium content in the spring was higher, the lower the mean annual rainfall of the district (Gar 1963). Sometimes the number of cases of selenium deficiency has been reported to increase during the use of land (Har 1961, Gar 1962). A reason for this may be a larger annual removal of selenium by the crop when the plant production is intensified.

In soils with a low selenium content other sources of selenium might add to the concentration in the surface soil, and they might play an important role in the supply to plants. Such sources could be precipitation, dust and fertilizers. An illustration of the size of these supplies is given in tables 1.4 and 1.5. More selenium is found in precipitation and dust in an industrial than in a non-industrial area as coal may contain some selenium (0.1 and 2 ppm have been reported (Lak 1967)). The content in fertilizers and other soil amendments varies much, for instance (table 1.4) by a factor of 50 for superphosphate and by much more for sulphur.

Table 1.4

Selenium content of some fertilizers and other soil amendments

Sample	Country	No. of samples	ppm Se	Ref.
Superphosphate	USA	8	<0.8 – 4.0	Rad 1935
	USA	10	0.4 – 3.9	Robb1970
	Europe		11 – 25	Sto 1922
	Denmark	2	5	Gre 1964
	Denmark	8	4.2 – 8.0	Gis 1971
	New Zealand	3	0.65 – 0.96	Wel 1966
PK	Denmark	12	3.6 – 5.5	Gis 1971
NPK (based on H_2SO_4)	Denmark	7	1.1 – 4.0	Gis 1971
NPK (based on HNO_3)	Denmark	8	0.02 – 0.2	Gis 1971
Natural phosphates	USA	54	<0.1 – 55	Rad 1935
	USA	14	0.7 – 178	Robb1970
	Europe, N. Africa, Asia	32	<0.8 – 55	Rad 1935
	N. Africa	8	3.1 – 25	Gis 1971
	S. Pacific Islands	4	1.0 – 2.6	Well 1966
Sulphur	S. Pacific Islands	4	0.01 – 1,950	Wel 1966
	Japan, Hawaii	37	4 – 29,000	Lak 1967
Carbonates	Japan, Hawaii	8	0.04 – 1.40	Wel 1966
Sulphates			<0.5	Gol 1954
$(NH_4)_2SO_4$			15 – 36	Sto 1922
	Holland	1	0.4	Wel 1966
$NaNO_3$	Chile	14	5.2	Rad 1935
	Chile	1	0.5	Wel 1966

Table 1.5

Selenium in water, air and dust

Source	Country	No. of samples	Se content	Ref.
Precipitation	Denmark	24	0.0001 - 0.0005 ppm	Gis 1968
Precipitation	Mass., USA		0.0002 ppm	Has 1967
Ground water	France	24	0 - 0.074 ppm	Mor 1965
Tap water and wells	Poland	105	0 - 0.004 ppm	Sik 1965
Dust in cities	USA	11	0.05 -10 ppm	Lak 1941
Air	Mass., USA	7	0.001 $\mu g/m^3$	Has 1967

1.1.3. Selenium Compounds in Soils

Most work concerning the content and the chemical forms of selenium in soils has been done because of a selenicity problem. The information about the forms of selenium in soils producing crops with an adequate or a low selenium content is more restricted owing to analytical difficulties and to the fairly newly arisen interest in these soils.

The oxidation state of selenium depends on the pH and the oxidation potential of the soil. In figure A.1 in the appendix the oxidation potentials of the various oxidation states are given as a function of pH. Most soils fall inside the pH range 4 to 9, and the redox potential is normally restricted to – 0.5 to + 1.0 volts (Kra 1956). In Holland potentials from –0.2 to + 0.5 volts were measured (Mar 1952). With an emperic method Lamm found potentials from + 0.3 to + 0.7 volts in 18 Danish soils (Lam 1964). The redox potential in a soil varies among other things with the water content and the temperature (McK 1960). Seasonal variations can be from 0.1 to 0.7 volts with the lowest potentials in winter and spring (McK 1960). Besides being done chemically, oxidation and reduction of selenium from various oxidation states can be done by micro-organisms (Shr 1967).

In acidic soils the normal oxidation state is + 4. The selenite ion precipitates with basic iron hydroxides with varying Fe:Se ratio, and the selenite forms absorption complexes with iron hydroxide (Will 1936, Cary 1967), but the true character of the compounds is not known. The equilibrium solubility of an artificial ferric selenite ferric hydroxide complex in soil has been determined by Geering et al. (Gee 1968) to 10^{-6} to 10^{-7} moles Se/l depending on the pH. The importance of complexes of this type for the selenium supply to the plants has also been considered by Watkinson (Wat 1962), who pointed to 0.45 ppm Se in this form in the soil as the lowest concentration for production of a crop sufficient in selenium. With an annual percolation of 600 mm and a selenium concentration of 10^{-6} moles/l, 4.5 μg Se/cm^2 would be brought into solution, i.e. all the selenium in a 10 gram soil sample

containing 0.45 ppm Se. Also aluminium hydroxide complexes may be formed in the soil, but they are probably less stable and more pH-dependent than the ferruginous compounds (Plo 1960). Selenites are also retained by clay, and this seems to play a role of the same importance as the formation of basic iron selenites.

Two examples may illustrate the role of iron and clay. On the Hawaiian Islands the seleniferous soils are non-toxic because the selenium is bound in an iron-rich zone (Bye 1938). In an investigation in New Zealand comprising very different soils Well found that the top soils contained on the average 0.60 ppm selenium, while the parent rocks only contained 0.42 ppm (Wel 1967). An accummulation of selenium was found in the B horizon, which is rich in iron and clay, and the average selenium concentration in part of this layer was 1.4 ppm. Unfortunately the pH in the many soil samples is not given.

In neutral to alkaline soils an oxidation of the selenite will take place if the soil is well aerated, and it will then be present in a leachable form. If an acidic soil layer is present below, the selenate may be reduced and retained there. Selenate does not precipitate with the cations in the soils, and it is not absorbed in iron hydroxides to the same extent as selenite (Will 1936, Bye 1938, Plo 1960).

Oxidation of elemental selenium apparently may occur both biologically and chemically (Sto 1922, Pet 1966, Gee 1968, Cary 1969). Organic selenium compounds are formed in micro-organisms and plants and will be released when the material decays.

Finally, volatile selenium compounds can be released from soils (Ganj 1958a, Abu 1968). In the most recent of the two studies the authors found that a few percent of the water-soluble native selenium in their soils (pH 7.7 - 7.9) was released during 20 days of incubation. Volatility may therefore play a role together with leaching for the amount of soluble selenium remaining in the top soil.

The total selenium content of a soil is rarely a useful figure for the prediction of the selenicity of the plants as it is exemplified in table 1.3. Attempts have therefore been made to evaluate the availability of the soil selenium. It has long been known that the most available forms are selenate (Hur 1937b) and organic selenium as has been shown with aqueous extracts of plants (Tre 1942).

The first determinations of plant-available selenium in seleniferous soils were based on water extracts. A water extract may be assumed to comprise all the selenate-selenium, the water-soluble organic selenium and the soluble selenite. The three fractions can be separated after a selective reduction of Se^{+4} because organic selenium is presumably non-reductable. This separation technique was first used by Williams and Byers (Will 1936). In seleniferous soils the water-soluble fraction accounted for about 0.3 to 50 % of the total

amount (Will 1936, Ols 1942, Fle 1957, Rav 1957). A large part of the soluble selenium was selenate (Will 1936, Ols 1942). But also appreciable amounts of organic selenium have been found (Will 1936, Beath et al. 1946, cited in Lak 1961). The water-soluble fraction is sometimes larger at depths of 30 to 90 cm than at the soil surface (Ols 1942, Rav 1957).

Though a large amount of water-extractable selenium results in a high uptake and even toxic concentration in the plants, this fraction is not a measure of the plant-available amount in a soil. In an attempt to find a more reliable method, Israeli workers have compared the amount of selenium soluble in other extractants and selenium isolated by an electro-decantation with the uptake by lucerne. Electro-decantation was found to provide the best expression for the amount of available selenium (Nis 1959). However, the method does not seem to have been used elsewhere. A more informative separation technique has later been developed by Cary et al. (Cary 1967) with the aim of separating the oxidation states from -2 to $+6$ of selenium from each other and from organic selenium. The method was meant for tracer experiments with soils with a low selenium content. The last method excepted, the attempts to determine the plant-available selenium do not seem to have been really successful. Furthermore, if radioactive selenium is not added, none of the methods is applicable to soils low in selenium because the concentrations in extracts will mostly be below the amount detectable by chemical methods.

1.1.4. Selenized Soils

In connection with an artificial increase of the soil selenium it is among other things necessary to know the rate at which the amount of selenium available to plants decreases. Besides removal by plants, the decrease may be due to leaching or fixation in the soil, but also volatility may account for part of the decrease. In recent years the fate of selenium added as selenite or elemental selenium has been followed in more detail (Dav 1966a, Cary 1967, Jon 1967, Cary 1969). In chapter 5 the fate of selenate, selenite and elemental selenium in Danish soils is described.

In field experiments it has been shown that the effect of added selenite upon the concentration in the plants had declined after one year by one to two orders of magnitude, but the decrease was small the second year. The removal of added selenite by the plants was 1 to 2 % during the first growth season (Gra 1965, All 1966, Dav 1966a and 1966b). In the field a retention of 65 % of the added selenite was found in the upper 7.5 cm and only 2 and 1 % respectively was found in the following two layers of 7.5 cm each (Dav 1966a). The remaining 30 % of the addition was not accounted for in this experiment. In a laboratory experiment (Jon 1967) Se-75-labelled selenite added on the top of soil columns was leached by adding water in amounts equivalent to 450 mm of precipitation. From 55 to 95 % of the selenite was

retained and calcareous soils retained more selenium than sandy and lateritic soils. Most of the selenium remained in the upper few cm of the column.

The leaching of barium selenate has been studied in columns with two alkaline soils (Brow 1969). More selenate was leached with a gypsum solution than with water. The same effect was seen when $BaSO_4$ was added to the column together with the $BaSeO_4$. The effect of the sulphates was explained by the authors as a result of the precipitation of Ba^{++} released from $BaSeO_4$. It would appear, however, that this explanation does not hold for the $BaSO_4$ addition.

The most detailed work on the conversion of added selenite and seleniur has been done by Cary et al. on mineral soils with pH-values varying from 5.4 to 7.8 (Cary 1967, Cary 1969). The two experiments demonstrated that a conversion of Se^{+4} and Se^0 into other oxidation states took place both when the soil was stored at −17°C, and when it was cropped with lucerne. Thus Se^{+4} added as $NaHSeO_3$ was converted into isotopically exchangeable selenite, into organic forms and into Se^0 and Se^{-2}. Part of the added Se^0 was refound in a readily soluble form and as exchangeable selenite, and, depending on the soil, only 30-80 % remained as Se^0 or other non-oxidized forms. The authors found that the concentration in lucerne was related to the readily soluble selenium in the soil (K_2SO_4-soluble), and that an increase of the soil pH lead to an increase of this fraction as well as of the concentration in the plants. Though the authors are rather conservative in their assumptions for the various fractions, they might still be less well defined than stated. Nevertheless, the results obtained are good indications of the size of the conversions, and the method offers good possibilities for comparison of soils.

1.2. Selenium in Plants

The concentration in the plants depends on many factors of which some of the more important are: the plant species, the development and the age of the plant, the source and the concentration of selenium, and the soil properties. In preparation for the possible use of fertilizers containing selenium it is interesting to investigate how the resulting concentration in the plants changes with time. Such a change is, of course, closely related to the above-mentioned chemical alterations in the soil.

With respect to the concentration in the plants it is natural also to mention briefly the transport and metabolism. This is done in the last subsection (1.2.3).

1.2.1. Selenium Concentration in Different Plant Species

The ability of plant species to accummulate selenium varies very much. A few species have been grouped as primary selenium indicator plants (or primary accummulators) because they are only found on toxic soils and contain much more selenium than non-indicators grown in the same soil.

Depending on the soil they contain from a few ppm Se to several thousands* (Bea 1934, Bye 1935, Will 1940). The most important indicators are some Astragalus species, some woody asters (Xylorhiza), some goldenweed species (Haplopappus) and some Stanleya species (the mustard family). These species are all found in the Western U.S.A. (Ros 1964). The only selenium indicator plant reported outside the U.S.A. is a Neptunia in Australia (Pet 1962). Indicator plants have given an increase in yield when selenite was added to the growth medium (Tre 1938). Some other plant species are characterized as secondary selenium accummulators because they are able to absorb more selenium than most other plants, but they are found on toxic as well as on non-toxic soils. The difference between the selenium absorber categories are illustrated by the following concentrations in plants (dried) grown on the same toxic soil: 5,500 ppm Se in an indicator, 300 ppm in a secondary accumulator and 23 ppm in grasses (Ros 1964). The selenium in decaying plant material has a high availability to plants. Hence the cultivation of such areas has often resulted in toxic crops.

The first comprehensive comparison of the selenium concentration in various agricultural crops and vegetables grown on non-toxic and non-selenized soils is presented in the present work (chapter 3). Other analyses have mostly been made on plants containing 10 to 500 ppm Se. Thus comparable data are available for crops from seleniferous soils in situ (Beath 1937, quoted in Ros 1964, Mox 1950) or potted (Fle 1962a), and from selenized soils (Hur 1937a, Ham 1963a, b, Ham 1964, Ehl 1968). As the relative concentration in the species seems rather independent of the experimental conditions, the data from one of the experiments (Fle 1962a) may illustrate the main features (table 1.6). As is the case with many nutrients, the lowest selenium concentrations are seen in the Graminiae, and the highest concentrations are seen in the clovers, onion leaves and in members of the Cruciferae family.

There is a tendency towards a higher selenium content in plant species normally rich in sulphur. Species known to contain much sulphur are for instance members of the Cruciferae and Lilliaceae families. Hurd-Karrer (Hur 1937a) found a very close relationship between sulphur and selenium concentration in plants grown on a selenized soil. As also in other reports the data in table 1.6 do not show quite the same degree of proportionality as that she found. The selenium concentration in a series of plant species is discussed in subsection 3.2.7 in relation to the sulphur concentration.

For the five species in table 1.6 where both the green part and the storage organs were analysed it is seen that the former contained at least twice as much selenium as the latter, just as is the case with sulphur. Similar results have been obtained on soils with added selenate or organic selenium (Ham 1964). For plants grown in culture solution supplied with selenite the root

*It may well be that some of the analytical results from the period 1930 to 1960 are too low owing to losses during drying and decomposition prior to the analysis.

Table 1.6

Selenium and sulphur content of farm crops and vegetables grown in a toxic soil containing 161 ppm Se. Pot experiment (Fle 1962a).

Plant species	Family	ppm Se in dry matter	ppm S in dry matter
Cock's-foot	Graminiae	28	2100
Perennial rye grass		34	2300
Italian rye grass		29	1700
Wheat, grain		39	1400
straw		40	2600
Oats, grain		39	2000
straw		40	2600
Barley, grain		35	1400
straw		42	2500
Lettuce	Compositae	56	3000
Artichoke, leaves		71	1700
roots		19	400
Parsnip, leaves	Umbelliferae	90	2900
roots		22	700
Carrot, leaves		29	3100
roots			900
Onion, leaves	Liliaceae	235	1900
bulbs		82	2100
White clover	Papilionaceae	153	4900
Red clover		103	4500
Pea, leaves		79	
shelled		9	3400
Radish, leaves	Cruciferae	145	
roots		35	2700
Cabbage, leaves		196	4600
Turnip, leaves		409	
roots		204	1900
Rape, leaves		203	5600

part, however, contains most selenium, presumably owing to reduction of the selenite to the elemental form and subsequent inhibition of the transport inside the plant (Hur 1934, Pet 1962, Broy 1966, Joh 1967). In cereals the grain contained less selenium than the straw (Ham 1963a) or about the same quantity (table 1.6 and Joh 1951). Correspondingly, the straw of many plants contains more sulphur than the grains or seeds.

The selenium concentration in crops grown on non-toxic soils (without any selenium addition) has mostly been determined in areas where selenium deficiency occurs or is suspected. The crops most often analysed are grain

and pasture species. In the U.S.A. lucerne has been chosen as an indicator of the selenium status in general because it is grown in most of the states, and because the concentration in lucerne is not affected much by variety and by the development of the plants. In the eastern and western states 68 % of about 700 samples contained less than 0.05 ppm Se in the dry matter (Kub 1967). Other results from the American survey have shown similar concentrations, which agrees with the heavy losses of livestock in those areas from nutritional muscle disease ,NMD, (All 1964a, Cart 1968, Cart 1970). In Sweden very low concentrations were found in grain and hay (table 1.7) which is in accordance with the widespread occurrence of NMD (Lin 1968). Results from a pot experiment with six Danish soils are shown in table 1.8. In this experiment clover contained less selenium than the rye grass. However, these data are in no way representative for the selenium concentration in these crops in Denmark.

Table 1.7

Selenium content in Swedish crops (Lin 1968 and Lin 1970)

Species	No. of districts	No. of samples	ppm Se in dry matter	
			Range	Ave.
Barley, grain	10	31	0.004 - 0.022	0.011
Wheat, grain	7	16	0.007 - 0.022	0.013
Oats, grain	9	26	0.005 - 0.046	0.016
Barley and oats, grain	1	14*)	0.005 - 0.020	0.012
Pasture species	5	19	0.006 - 0.064	0.025
Beet pulp	1	4	0.044 - 0.310	0.17

*)Samples from seven farms in the Uppsala district where NMD and liver dystrophy are known to occur.

Table 1.8

Selenium concentration in plants grown in Danish soils. Pot experiment (Gre 1964)

Soil	ppm Se in dry matter		
	Rye grass	Red clover	White mustard
Rise	0.4	0.20	0.5
Lammefjord	0.24	0.17	0.33
Ødum	0.2	0.07	0.13
Blangstedgaard	0.06	0.04	0.05
St. Jyndevad	0.08	0.03	0.07
Borris	0.06		
Average	0.17	0.10	0.22

1.2.2. Factors Influencing the Selenium Concentration in a Plant

A growing interest in an increase of the selenium concentration in the plants has initiated the search for a suitable compound, and a compound effective for a whole season or more has been the purpose. From early experiments the relative availability of some selenium compounds is known. These experiments were mostly made with pot or water cultures. It has been found that selenite-Se is far less readily available than organic Se from an aqueous extract of an indicator species (Tre 1942), that selenates (the sodium and calcium salts) as well as organic Se are available to a much greater extent than Na_2SeO_3 and $Fe(OH)SeO_3$ (formula not exactly known), and that FeSe, and to an even greater extent elemental selenium, was of limited availability (Mox 1950, Ganj 1958b). Only Beath et al. (Bea 1937) found a considerable utilization of elemental selenium by indicator species of Astragalus while in a tracer experiment with pasture species Peterson and Butler (Pet 1966) found up to 0.5 % in total uptake over a five-month period when extremely small additions of elemental selenium to the soil were used.

In a field with mixed pastures the effect of a top-dressing with Na_2SeO_4 or Na_2SeO_3 was followed (Gra 1965). In the first harvest the concentration in the plants was five to twenty times higher after selenate addition than after addition of selenite. However, the effect of selenate declined faster than that of selenite, and after one year the effects of the two salts were comparable. The selenium concentration in the plants was roughly proportional to the selenite or selenate addition (Gra 1965, Dav 1966a) when the first cut was disregarded. The amounts added were from 70 to 1120 g Se/ha which would correspond to about 0.02 to 0.3 ppm Se if it were distributed in the plough layer. About 0.2 ppm Se was found in pasture species three to four months after top-dressing with 280 g Se/ha as selenite (Dav 1966b, Wat 1967). After two years the concentration in the plants had decreased to two to four times above that in the control, which contained about 0.01 ppm.

As observed in pot experiments (Mox 1950, Ganj 1958b) no difference in utilization of readily and slightly soluble selenites was found (Wat 1967). To overcome a high initial foliar and root uptake the authors tried a lead silicate-sodium selenite frit which caused a more moderate initial concentration in the herbage. Others (Cart 1969) have used ferric hydroxy selenites, but they did not offer much advantages compared with soluble selenites. After top-dressing with elemental selenium both Grant (Gra 1965) and Watkinson and Davies (Wat 1967) found an increase in plant selenium in the first cuts, but this could have been due to foliar contamination. However, also deep application of elemental selenium caused a small increase (Cart 1969). The general conclusion to be drawn from the field experiments was that a selenite salt was to be preferred, but none of the selenites tried was ideal.

Besides the properties of the compound at the disposal of the plants, a number of other factors such as soil amendments and changes in the climate may influence the absorption by the plants.

The possibility of diminishing of the absorption by means of other compounds has been considered for toxic soils. Thus as first shown by Hurd-Karrer sulphate reduces the absorption of selenate (Hur 1933, Hur 1938). On seleniferous (mineral, alkaline) soils in Israel sulphate reduced the concentration in lucerne by 60 to 90 % (Rav 1959). Correspondingly, in plants from an Irish seleniferous soil (highly organic, neutral) Fleming (Fle 1962b) found a reduction in the concentration when superphosphate was added. The effect was ascribed to the sulphate content, and this was later confirmed (Fle 1966). Whether the selenium was present as selenate in these soils is, however, not known. If the soils are already saturated with sulphate, a further addition is without any effect (Bea 1937, Fra 1937b). Sulphate does not prevent the absorption of selenite-Se from culture solution (Hur 1937b) and according to some authors possibly not from soil either (Dav 1966b). It has recently been found that selenite added together with a NPK-fertilizer resulted in plants with a higher Se concentration than if selenite was added together with a PK-fertilizer (Gis 1971). The addition of barium chloride to seleniferous soils in Israel reduced the selenium concentration in lucerne perhaps through a precipitation of $BaSO_4$ and $BaSeO_4$ (Rav 1959). A determination of the nature of the selenium in these soils would have been valuable. On the Irish soil barium chloride had no effect (Fle 1962b).

In consequence of the high availability of organic selenium the effect of water-soluble proteins upon the absorption of selenite was investigated (Tre 1942). An increase in the absorption was found, and the authors concluded that soils rich in nitrogeneous organic material would allow a greater selenium accumulation in the plants.

An increase in the soil pH also meant an increase in the selenium concentration in plants in a pot experiment with soils supplied with selenite (Cary 1969). The results agree with the increased amount of readily soluble selenium in the soils with $CaCO_3$ added (see 1.1.4).

The possibility of a dependence of the selenium content of crops upon the temperature and precipitation has only been considered by a few authors. In early investigations with the deep-rooted Astragalus species diverging results were obtained (Ros 1964). In Ostrobothnia NMD in pigs (Lann 1960) occurs, especially when the harvest season has been rainy and cold. A lower selenium concentration in crops from rainy and cold periods would agree with the behaviour of many nutrients (Stee 1965). But the cause of the increased occurrence of NMD might as well be a decrease, due to the weather, in the content of linolenic acid and in vitamin E in connection with an already low selenium concentration (Oks 1965).

The variation of the selenium concentration with the development of the plant is only partly known. For some plants rich in selenium the concentration decreases with increasing maturity of the plant (Mox 1950, Ros 1964). Though the total contents are not given, the decreases are in some

cases so large that a gaseous loss might have occurred. For oats and barley grown in the field a concentration increasing with the development was found, most pronounced in the grain (Lin 1968). Also Lane and Fleming (Lane 1966a), using rye grass in pot culture, found an increase in selenium concentration with the development. If for the agricultural crops the highest concentration is generally found in the old plants, this will be in contrast to what is the case with most macro- and micronutrients (Stee 1965).

A series of Danish experiments elucidating the influence of some of these factors (soil type, Se-compound, other additions) are treated in chapter 4. The influence of precipitation is commented upon in section 3.2.

1.2.3. Selenium in the Metabolism of the Plant

For selenium to be considered an essential ultra-micronutrient it must be necessary for the growth and the reproduction of the plant, and it must play a role in the metabolism (Arn 1939, Nic 1961). The first condition seems fulfilled by Se-indicator plants as they respond to selenium supply when they grow with no or small amounts of the element present (Tre 1938). A response from other plants has sometimes been reported (Bee 1961). In a water-culture experiment Broyer et al., however, found no stimulating effect of selenite on the growth of lucerne and clover, and they concluded that a possible selenium requirement of these two species is below 0.08 ppm in the dry tissue (Broy 1966). As for the metabolism, several seleno amino acids have been identified in higher plants (Horn 1941, Tre 1960, Pet 1962, Shr 1965, Vir 1965), but their role is not known. The necessity for reproduction has apparently not been investigated. Hence it is not clear whether the requirements for essentiality are fulfilled even for the indicator species. Further, there seems to be the possibility that the response of indicator species could be caused by a phosphate intoxication hindered through ion antagonism between PO_4^{---} and SeO_4^{--} or SeO_3^{--} (Shr 1969). If, however, selenium is essential to some species, it seems probable that it should be essential to all higher plants.

Increasing of the amounts of selenate or selenite in the growth medium sooner or later results in a depression of growth (Ros 1964). Even indicator species may be depressed in growth, especially from selenium in plant extract (Tre 1938, Tre 1944). Intoxication by selenate manifests itself by stunting of the roots and by chlorotic leaves, while intoxication by selenite appears from darker leaves and from pinkish roots caused by reduction of selenite to the elemental selenium (Hur 1937b).

Owing to the similarities between sulphur and selenium the transport and the metabolism in the plants of the two elements could be expected to follow almost the same routes.

Sulphur is predominantiy taken up by the roots as sulphate; selenium as selenate, selenite or as organic selenium. Thus sulphate and selenate might

compete with each other during the absorption. After addition of selenate a decreased absorption rate for sulphate has been found with excised barley roots (Leg 1956) and with isolated leaves (Kyl 1960). In the leaves the incorporation of sulphur into proteins was measured too, and it was found that the incorporation was inhibited more than the absorption (Kyl 1960). In studies performed by Hurd-Karrer (Hur 1938), however, an increase in the sulphate absorption by wheat plants was seen when the selenate concentration in the nutrient solution was increased. This phenomenom is treated in section 6.2.

For a first study of the incorporation of selenium into metabolic products the oxidation state of selenium in various fractions of plant extracts has been determined. An aqueous extract mostly contains from 50 to 100 % of the plant selenium (Bea 1947, Ham 1963a, b, Ham 1964), while an extraction with 80 % ethanol released 15 to 95 % (Pet 1962). In a water extract of some agricultural plants and of some Astragalus and Stanleya species all the soluble selenium was in organic form, while in a series of other accumulator species both organic Se and selenate-Se were found in the extract (Bea 1947). Trelease and Beath (1949, quoted in Ros 1964) found that the form of selenium in maize depended on the selenium source. When the source was organic Se or selenite, only organic Se was found in an extract of the plant top, while a supply with selenate resulted in the presence of both selenate and organic Se in the top. In the studies performed by Hamilton and Beath (Ham 1963a, b, Ham 1964) the ratio between organic and inorganic selenium in the plant extract seemed independent of whether the supply was organic Se or selenate-Se. Significant amounts of selenite in a plant extract have only been found by Peterson and Butler (Pet 1962). In a water culture experiment with 5- to 6-week old plants they found selenite in the plant tops after ten days of growth in the presence of selenite. In section 4.5 the oxidation states of selenium in radish grown in the presence of selenate or selenite are given.

Despite the search for selenium analogues to the sulphur amino acids only a few have been found, but lack of sulphur analogues to some of the known seleno-amino acids has also been found. Attempts to isolate and identify the seleno-amino acids have until now resulted in about ten acids (Horn 1941, Tre 1960, Pet 1962, Spå 1964, Shr 1965, Vir 1965, Jen 1967, Pet 1967, Nig 1969). In the Se-indicator plants the dominating seleno-amino acid is Se-methyl-selenocysteine, while Se-methyl-selenomethionine occurred as traces only (Shr 1965). In the non-accumulator species of Astragalus the predominant seleno-amino acid is Se-methyl-selenomethionine (Vir 1965). Whether this difference between indicator and non-indicator Astragalus species is more general is not yet known. The metabolic role of Se-methyl-selenocysteine is not known, but Virupaksha and Shrift (Vir 1965) suggest that "methylation of selenocysteine represents a detoxification mechanism whereby excess selenocysteine is prevented from being incorporated into proteins".

The metabolisms of sulphate and selenate are definitely not identical, but all the steps, especially in the selenate reduction, are still not known. The studies of Shrift and Virupaksha (Shr 1965, Vir 1965) may serve as examples of the differences in the metabolic routes. Among other things they found that though a non-accumulator plant contained Se-methyl-selenocysteine, the sulphur analogue was not found (Vir 1965). Wilson and Bandurski (Wils 1958) observed that the sulphate-activating system in yeast could form APSe (adenosine-5'-phosphoselenate). The reaction is catalyzed by ATP-sulphurylase (ATP = adenosine-tri-phosphate), an enzyme that is active with all group VI anions. However, while APS (adenosine-5'-phosphosulphate) forms PAPS (3'-phospho-adenosine-5'-phosphosulphate) in the presence of APS-kinase, the higher plants cannot form PAPSe (3'-phosphoadenosine-5'-phosphoselenate). APSe was supposed to be cleaved into SeO_4^{--} + AMP (adenosine-5'-phosphate) by hydrolysis (Wils 1958). Nissen and Benson did not find selenium analogues of choline sulphate, flavonoid sulphates and sulpholipid and explained this as inability of the plant to form PAPSe (Nis 1964).

Release of gaseous selenium compounds from seleniferous vegetation was recognized very early owing to a characteristic odour (see Ros 1964). In recent investigations release of volatile selenium compounds during growth has been found both in Astragalus racemosus (an indicator plant) and in lucerne (Lew 1966, Ash 1967, Eva 1968). One of the compounds released from Astragalus racemosus was identified as dimethyl diselenide (Eva 1968). An absorption by plants of volatilized selenium compounds has also been measured (Broy 1966, Ash 1967).

1.3. Selenium in Animal Nutrition

The history of selenium in animal nutrition has two important periods, the 1930's and the late 1950's. In the first period it was discovered that a high selenium content in the food was the cause of intoxication of animals in certain areas of the U.S.A. Much work was done to elucidate the problem, especially in South Dakota and Wyoming where such incidents were widespread with severe economic losses to the farmers. The second important step forward was the discovery in 1957 of the probable essentiality of selenium to higher animals. Selenium deficiency in agriculture was first observed in New Zealand, and many contributions demonstrating the effect of selenium are from this country.

1.3.1. Toxicity

Only very few of the common trace elements may appear in the food in amounts toxic to higher animals. Selenium is one, molybdenum and nickel in restricted areas are two other examples, and recently mercury due to pollution has been added to the list.

Until selenium was known as the true cause, some Astragalus species were, among other plants, associated with the intoxication in livestock. Sometimes also toxic wheat was found. Upon request Robinson (Robi 1933) analysed wheat for selenium, and he demonstrated that two samples of toxic wheat contained 5 and 10 ppm selenium, whereas non-toxic wheat did not contain selenium in a detectable amount, and soon the role of selenium was established. In this period it was also found that certain plant species can accumulate hundreds or thousands of ppm of selenium, and the terms Se-accumulator and Se-indicator were introduced. The protein-bound selenium in seleniferous grains and grasses produces the alkali disease characterized by lameness, elongation of the hoofs and loss of hair. The water-soluble selenium in indicator plants and salts of selenites and selenates produce blind staggers and subsequent emaciation and neuromuscular complications (Ros 1964). The relative toxicity of seleniferous plants depends on the plant species, selenium in wheat being one of the more poisonous sources.

A selenium concentration of about 5 to 10 ppm in the fodder will produce chronic poisoning in domestic animals (Fra 1938, Mox 1943). In a newer investigation 1 ppm in the diet was found to influence the health of chickens (Rei 1958). The lethal dose of orally administered selenite-selenium is 1 to 10 mg per kilogram of body weight (Mil 1940, Ors 1960). A comparison of diets containing 25 and 50 ppm respectively of various elements resulted in the following order of increasing toxicity: $As^{+3} < Te^{+4}, Te^{+6} < V^{+5} < Se^{+4}, Se^{+6}$. The elements were supplied as sodium salts (Fra 1937a).

Selenium poisoning in livestock has played an important role on the Great Plains in the U.S.A., but poisoning also occurs in Ireland, Israel, South Africa, Australia, and South America. In man selenium poisoning has been observed in the U.S.A. (Lemley and Merryman 1941, quoted in Ros 1964) and in Columbia, South America (Benavides and Mojica 1959, quoted in Ros 1964).

1.3.2. Deficiency Symptoms

The discovery of the importance of selenium for animal health was complicated because vitamin E, selenium and sulphur amino acids may to some extent replace each other. Klaus Schwarz, U.S.A., found that in rats liver necrosis developed when they were fed a Torula yeast diet alone. The necrosis was prevented by cystine or vitamin E. On another American yeast diet it was not possible to induce the necrosis, and Schwarz therefore assumed that this yeast contained a third compound preventing the disease. This compound was called Factor 3 (Schw 1951a, b). Other biological material also contains Factor 3, e.g. kidney (Schw 1959). Attempts to purify the biologically active compound led to the discovery that it contained selenium, and that the biopotency was proportional to the selenium content (Schw 1957). The effect of Se^{+4} was 500 times that of vitamin E and 25,000 times

that of L-cystine. Dam et al., also in 1957, found that muscle degeneration and exudative diathesis in chicks were prevented with selenium (Dam 1957a, b). Besides it was shown that the preventing effect of cystine was due to a selenium impurity (Schw 1959). Liver degeneration in rats is prevented either with 0.7 μg Se per 100 g diet as Factor 3 selenium or with 2.0 to 2.5 μg Se per 100 g diet as selenite, selenate or seleno-amino acids (Schw 1958).

After these discoveries the extent to which selenium prevents other diseases was studied, primarily diseases only partly prevented by vitamin E administration. The result of numerous investigations with ruminants, pigs and chicks is that selenium is now considered an essential element to higher animals.

In cattle, lambs and pigs an important selenium-responsive disease is degeneration of muscles. Poultry may also be affected by this deficiency. Because the muscles become white (calcification), the disease was called white muscle disease, WMD. Today, with the recognition that it is a nutritional deficiency, it is mostly called nutritional muscle disease, NMD. The disease is accompanied by a low weight gain. NMD-affected animals have been found in New Zealand (Har 1961), in Australia (Gar 1962), in the U.S.A. (Kub 1967), in Finland (Oks 1965), in Sweden (Gra 1958, Lann 1960, Ors 1961, Tan 1965, Lin 1966), in Denmark (Rasb 1965), and in some other countries. In connection with NMD a low selenium content in the food has been found whenever it was analysed, and the affected animals were found to respond to a supply of selenium (Har 1961, Old 1963, Oks 1965) or of selenium plus vitamin E (Lann 1960, Lin 1966). In Denmark some effect of selenium upon muscle degeneration in pigs has been observed (Lud 1964).

Other selenium-responsive diseases are selenium-responsive unthriftiness (SRU) known in sheep and cattle and selenium-responsive infertility (SRI) which is common in ewes. These two diseases are widespread in New Zealand, and selenium treatments have been successful (Har 1961).

In the industrial countries selenium deficiency is not to be expected in man because of the large variations in the food as compared with that of the animals. But in malnourished infants with the Kwashiorkor disease (a protein deficiency) selenium supply has been advantageous with respect to the weight gain (Schw 1961, Hop 1967).

The selenium requirement of different animal species is not well known. It depends on the vitamin E content of the fodder, but also on the content of unsaturated fatty acids, and probably also on other components in the food with which there may be a metabolic interaction. A selenium concentration on a dry basis below 0.02, 0.03 or 0.05 ppm in the pastures has caused NMD or SRU in sheep and cattle (Har 1961, Old 1963). Gardiner and Gorman (1963) consider 0.05 ppm Se in the diet necessary as they have found NMD cases in sheep at a concentration of 0.04 ppm. In the U.S.A. 0.1 ppm Se in the pastures appears to be the boundary between areas with and without

NMD cases in lambs and calves (All 1964a), while in Sweden 0.03 ppm seemed to be a more likely limit (Lin 1970).

From the above it seems reasonable to consider 0.05 to 0.1 ppm Se in the food the minimum desirable concentration. Consequently the conclusion is that a selenium concentration in the dry foodstuff of 0.1 to 0.3 ppm will prevent deficiency symptoms without having toxic effects. The maximum concentration tolerable is probably 1 ppm.

Possible countermeasures against selenium deficiency are injections into each animal, administration of selenium with the diet or raising of the concentration in the crops by addition of selenium to the soil. The first two methods of dosage have been used successfully (Beh 1962, Ors 1963, Wol 1963, Løn 1965). However, the therapeutic as well as the prophylactic treatment of animals decided for comsumption is restricted in most countries by the health authorities, and only in New Zealand is it allowed to add a limited amount of selenium to the fodder (Fro 1967). Hence the interest concentrates on the possibility of an enrichment of the crops through an addition to the soil.

1.3.3. Selenium in the Organism

Selenium is widely distributed in the fluids and tissues of the organism. The highest concentrations are found in the kidney and the liver. Selenium is excreted in the urine and the faeces, and at higher intake it has also been measured in the expired air.

The distribution and the concentration of selenium in tissue of healthy and Se-deficient lambs and pigs are illustrated in tables 1.9 and 1.10 respectively. The data in table 1.9 refer to fresh weight, while those in table 1.10 refer to dry matter. By far the highest concentration is found in the kidney, but the liver is considered more suited for diagnostic purposes (And 1964). Some investigations have pointed to a concentration of 0.2 ppm in

Table 1.9

Selenium concentration in liver, kidney and blood of normal lambs and lambs affected with congenial WMD or SRU (Har 1967). ppm Se on fresh-weight basis.

Area	Age of the lambs	No. of animals	Kidney	Liver	Blood
Unaffected	newborn	20	0.65	0.210	
Affected with WMD		25	0.20	0.052	
SRU not recognized	6-9 months	40	1.1	0.16	0.06*)
SRU suspected		25	0.82	0.10	
SRU occurs		55	0.20	0.02	0.01**)

*) 76 animals, **) 45 animals.

Table 1.10

Selenium concentration in tissue of normal and NMD affected pigs, weight about 70 and 40 kg respectively. Six pigs in each group. The food contained 0.126 and 0.021 ppm Se respectively (Lin 1968). ppm Se in dry matter.

	Kidney	Liver	Skeletal muscle	Heart	Pancreas	Spleen	Lung
Healthy	11.5	1.8	0.5	1.1	1.4	1.3	1.1
NMD affected	2.5	0.2	0.2	0.2	0.2	0.4	0.3

the dried liver of lamb as the limit below which deficiency is likely to exist (Cou 1961, All 1966). From the data in table 1.10 this value seems too low for pigs. In healthy Danish cows a concentration in the dried liver of 0.7 ppm Se was found, but a lowest permissible level was not established (Bis 1970).

The distribution of the excretion between urine and faeces depends on the dose and the animal species; thus in monogastrics the main excretion is via the urine, in ruminants via the faeces (But 1961, Cou 1961, Gant 1965). An important difference between monogastrics and ruminants in their absorption of selenium given perorally was demonstrated by Wright and Bell (Wri 1966). They found that after an oral dose (about 0.4 ppm Se in the diet) only about 35 % of the selenium was absorbed by the sheep, while 85 % was absorbed by the swine. This difference in selenium absorption was considered the reason for a more frequent occurrence of Se-deficiency in ruminants than in monogastrics.

Part of the selenium is retained so long in the body that a supply to the mother animals during or even prior to gestation will protect the young because selenium passes into the placenta and is excreted in the milk (McC 1948, McC 1964, Wri 1964, All 1966).

The metabolism of physiological amounts of selenium and its function has been studied in many experiments (reviewed in Mut 1967), but is still far from being understood. Contrary to sulphur, selenium is mostly following reductive pathways and is built into organic compounds in the organism. The chemical form of the excreted selenium depends on the route. In the exhalation selenium is found as dimethyl selenide (McC 1952); in the urine selenium is present as inorganic selenium and in forms associated with the ethereal and neutral sulphur fractions (Gant 1965); in faeces of ruminants part of the selenium is found in an insoluble, inorganic form (Cou 1961), presumably as insoluble metal selenides or in the elemental form (But 1961). In milk selenium is closely connected to the protein fraction (McC 1948, McC 1963).

Seleno-methionine is a stronger lipid antioxidant than methionine, and it can decompose lipid peroxides (Olc 1961). Also selenoprotein may act as a

lipid antioxidant (Bie 1961). Tappel (Tap 1967) considers the role of vitamin E that of an antioxidant solely, which is in accordance with the opinion of many other workers. The difference in activity of selenium and vitamin E against various deficiency symptoms is then explained by Tappel as a question of the character of the phase where the antioxidative action is required. If the phase is lipid, the active compounds are tocopherols, if the phase is aqueous, the active compounds are seleno amino acids. According to Schwarz, however, selenium and vitamin E might be independently protective factors, catalyzing different, alternate pathways of metabolism. Vitamin E might play a role in the lipoyldehydrogenase system, while selenium might have a functional site in the decarboxylation reaction (Schw 1965). Later experiments by Tappel and coworkers showed that selenocystine catalyzes sulfhydryl-disulphide exchange reactions and forms a complex with the sulfhydryl groups in some enzymes whereby these are protected against oxidation (Dic 1969). According to Horwitt the many different symptoms of vitamin E- and of selenium-deficiency may be explained by animal species, age, the polyunsaturated fatty acids in the diet and other variables rather than by multiple functions of the compounds (Horw 1965).

1.4. Selenium Balance

In the previous sections the various links in the selenium movement from soil to plant and to animal have been discussed with respect to chemical or physiological behaviour in each of the media. The distribution between the amounts transported along the various routes vary with the soil properties, the climate, etc. In Denmark the yearly supply can be 1.2 g and 0.8 g Se/ha from precipitation and fertilizers. This estimate assumes per year 600 mm of precipitation (0.0002 ppm Se) and 400 kg/ha of superphosphate (2 ppm Se). The use of an NPK fertilizer instead will mean a lower selenium supply. For comparison an average crop will remove from 0.4 g (barley) to 3 g (swedes) selenium per hectare a year under Danish conditions (see chapter 3.2). At present only a rough estimate can be given of the concentration in plants resulting from an addition of a selenite salt to a soil, and only for the first few months after the addition. Only a few of the amounts transported (or immobilized) along the routes are known, and that only in a small number of soils. Thus many questions must be answered before an addition of selenium to Danish soils could be recommended.

2. METHODS

In the course of the present investigation, methods were needed for the determination of total selenium and sulphur in plants and soils. For some materials knowledge of the oxidation state of the selenium was also necessary. The chemical techniques, based largely upon the use of nuclear methods, are described in this chapter. In spite of the large number of methods already in existence it became necessary to make a number of adaptations, mainly to make the methods applicable to agriculture. A particular problem was presented by the volatility of some of the selenium compounds in biological material, and a control of all steps from drying to the chemical separation was required to ensure that no loss occurred.

2.1. Drying

Selenium can be released by drying. Thus Beath et al. (Bea 1937) found a loss of up to 60 % after air drying of some indicator species, while no loss occurred from grain crops, grasses or vegetables. The same authors found that the loss from a seleniferous grass was negligible after drying at 50 to 60°C for 48 hours. Until recently no other investigation of losses from plants at different temperatures has been made. Meanwhile, many investigators cautiously used drying at temperatures below 45°C and often in a draught of air (Wat 1960, Gra 1963, Broy 1966, Dav 1966a). Taussky et al., working with biological tissues and fluids, preferred freeze drying (Tau 1966). Drying at room temperature might further rottening and thus offer a new possibility of selenium loss.

More recently the problem was reconsidered. Using Se-75 and drying at 70°C for 24 to 48 hours Asher and coworkers (Joh 1967) found in lucerne and clover that the release was 1/2 to 1 % of the plant selenium; for species containing more selenium the release was higher; thus the largest loss, 7.6 %, was found in Astragalus roots. Watkinson (Wat 1966) found in non-seleniferous plants that drying at 100°C for six hours caused a 2 % loss, while after 18 hours the loss was 6 %; nevertheless, Watkinson in his experiments used drying at only 40°C. Finally, Ehlig et al. (Ehl 1968) have measured a loss from 0 to 5 % when drying fresh leaves of about twenty species at 70°C for 30 hours.

At the outset of the present investigations the losses from selenium salts and from plant material were studied. From table 2.1, which gives results for selenium salts, one can see that selenite and selenate salts gave no losses but that for selenium dioxide, selenium was released from the silica tube at 100°C when dryness had been reached. If, however, the selenium dioxide was placed on a piece of filter paper, the loss at 100°C was much less. In the

Table 2.1

Stability of selenium salts during drying.*)

1 μg Se as	Drying time, hours	In silica tubes			On filter paper	
		50°C	70°C	100°C	70°C	100°C
SeO_2	0	100	100	100	100**)	100**)
	1	96.6	100.0	100.7**)		
	3	98.0	98.2**)	84.9	101.6	99.0
	6	96.6	96.8	65.7		
	13			39.8		97.8
	24	97.4**)	99.4		99.5	93.2
K_2SeO_3	0	100	100	100	100**)	
	3	98.0	100.8**)	98.6**)	98.8	
	24	98.0**)	100.0	98.6	100.0	
K_2SeO_4	0	100	100	100	100**)	
	3	100.0	100.9**)	99.3**)	99.5	
	24	98.0**)	98.7	98.6	100.0	

*) One μg Se containing radioactive tracer was placed as a 100 μl aqueous solution in the test vial.

**) Indicates that dryness has been reached at this point.

preparation of reference samples, SeO_2 in silica tubes was dried at 60°C, while SeO_2 on filter paper was air dried.

A few plant species containing 1 to 10 ppm Se-75 labelled selenium were dried at 30°, 50°, and 70°C for 24 h. No difference in radioactivity was found. Nor was a loss of selenium found at temperatures below 50-60°C in a later investigation at this laboratory (Gis 1970a).

For the present purpose it was decided to dry the plant material as quickly as possible at 50°C in a ventilated oven. Thick leaves, stems and roots were cut before drying to shorten the drying time, and the material was spread in a thin layer in a tray. Beets and potatoes were ground to a pulp before a sample was taken for drying. Cereals and mustard were air dried before threshing, and only dried for a short time at 50°C before the selenium analysis. Except for grains and seeds the dried material was ground and mixed before storage.

The soil samples were mostly stored in plastic bags without previous drying. When needed, soil samples were air dried.

2.2. Storage and Heat Stability

Loss of selenium from seleniferous cereals during year-long storage has been reported (Mox 1938). Losses of up to 60 % over a 3 to 5-year period were found, but the authors considered a short period in a badly ventilated and sun-heated room to be the main cause of the loss.

Table 2.2

Loss during storage of selenium from plants containing 7 to 50 ppm Se labelled with Se-75. Grains and seeds were stored as such, the other samples as briquettes.

Plant species	Months from harvest to		Second measurement in per cent of the first
	1st measurement	2nd measurement	
Red clover, 1st cut	1	11	97.5
— 3rd cut	1	7	101.0
Barley, green	1	11	99.2
— grain	3	9	101.0
— straw	3	9	100.8
White mustard, green	1	11	99.4
— — seed	2	9	103.6
— — straw	3	9	101.2
Average			100.5

Table 2.3

Loss of selenium during storage controlled by repeated activation analysis. The plants were stored as hay or as powder.

Plant species	Months between analyses	ppm Se		2nd analysis in per cent of the 1st.
		1st anal.	2nd anal.	
Rye grass	7	0.135	0.134	99.2
—	5	0.23	0.213	92.2
—	24	140.9	126.2	89.7
—	24	153.2	139.3	91.0
Lucerne	7	0.137	0.139	101.5
—	7	0.187	0.197	105.5
—	24	4.82	4.70	97.5
—	18	2.19	2.07	94.9
Mustard, straw	18	3.91	3.82	97.6
Oats, —	18	3.08	2.90	95.6
Rye, —	18	4.34	4.22	97.0
Sugar beet, top	16	1.62	1.49	92.3
— — , beet	9	0.66	0.62	94.0
Average	15			96.0

At the start of the present investigation no information seemed available about storage loss from other species or from species with a low concentration of selenium. Normally from one to eight months passed between harvest and determination of selenium. In the mean time the samples were stored in glass vessels, plastic boxes or plastic bags in a cellar or in a labor-

atory room to avoid exposure to sunlight. No loss was observed in samples of red clover, barley and mustard stored as briquettes under these conditions for periods up to 11 months (table 2.2). In another series comprising more plant species with selenium contents from 0.1 to 150 ppm and stored as hay or powder, analyses performed at 5 to 24 months' intervals showed an average of 4.0 % decrease (table 2.3). The greatest decrease was about 10 % and was found in highly seleniferous rye grass stored for two years. Owing to the experimental errors the stability of the different species with respect to selenium content cannot be compared. A third control at this laboratory gave similar results. The greatest loss was 13 % in hay of red clover and 10 % in rye grass powder after nine months of storage, while the loss from briquettes was about 1 % in red clover and lucerne and 7 % in rye grass (Gis 1970a).

In connection with the selenium analysis the samples were irradiated in the reactor for up to 24 periods of six hours each. During these periods the temperature in the samples did not exceed 45°C. Whether a selenium loss at this temperature is likely to occur over such a long period was investigated with Se-75 labelled material (table 2.4). The seeds and grains were retained as such, the other samples were pressed into briquettes, i.e. all samples were kept in the same form as during an irradiation. After 230 hours at 50°C the temperature was increased to 100°C for 24 hours. No appreciable losses were found.

Table 2.4

Heat stability of selenium in dried plant material expressed in per cent of the activity before the heat treatment. Se-75-labelled material containing 7 to 50 ppm Se.

Plant species	Hours at 50°C		Additional 24 h at 100°C
	18	230	
Red clover, 1st cut	97.6	96.9	96.9
— — , 7th cut	98.6	99.8	101.7
Barley, green	99.8	101.4	95.4
— , grain	96.0	97.4	101.5
— , straw	99.7	98.4	99.7
White mustard, green	98.2	102.3	93.8
— — , seed	99.7	101.2	102.9
— — , straw	97.1	96.4	96.7
Average	98.3	99.2	98.6

The conclusion is that loss of selenium during storage may occur, and it is more likely to occur in samples with a large surface (like powder) than in pressed material, but in most cases the loss will not exceed 5 % in a year. A loss of this magnitude will be of no importance to the conclusions of this investigation.

2.3. Selenium Determination

In the present material selenium concentrations as low as 0.01 ppm in the dry matter could be expected. Only two methods have sufficient sensitivity for this purpose: neutron activation analysis and fluorometric determination with either 3,3′ diaminobenzidine (DAB) or 2,3 diaminonaphthalene (DAN). For the determination of high selenium concentrations several methods are usable; atomic absorption is a convenient choice for routine analysis.

The fluorometric method is based on the formation of a piazselenol when selenium reacts with DAB or DAN. According to Watkinson (Wat 1960) 0.02 μg Se can be determined with DAB. Cousins (Cou 1960) reported a similar sensitivity, but considered 0.1 to 2 μg Se a more adequate range for routine work. The use of DAN as suggested by Parker and Harvey (Par 1962) improved the sensitivity so much that the minimum detectable amount is 0.0005 μg (Wat 1966). The fluorometric methods with various modifications (Dye 1963, Gra 1963, All 1964b, Lane 1966b, Mol 1967, Ewa 1968, Hof 1968, Lev 1971) have been widely used for determination of low levels of selenium in biological materials. However, the overall recovery of selenium may be only 75 % (Kel 1961). To control the loss some investigators have inserted an isotope dilution in the procedure (Kel 1961, Cuk 1964, Lin 1965).

The neutron activation analysis has a sensitivity one to two orders of magnitude above the fluorometric method. The principle is that after bombardment with neutrons the resulting radioactive isotope can be measured. Besides the greater sensitivity, the advantages of activation analysis compared with fluorometric analysis are several. Most important is that the method is selective, and that after irradiation all reagent-contamination problems are eliminated, otherwise severe problems in conventional chemical determination of tracer amounts. Another important advantage is that after irradiation a macro-amount of selenium can be added. As soon as complete isotopic exchange has taken place, any loss of selenium can be controlled by a determination of the chemical yield. Neutron activation has been used for the determination of selenium either alone or together with other elements in soils and rocks (Kur 1962, Bru 1967), in plants (Bow 1963a, Oks 1965, Stei 1967) and in animal tissue and fluids (Ledd 1961, McC 1961, Koc 1962, Bow 1963a, Gui 1963, Wes 1965 a and b, Sam 1967, Lun 1970). Activation analysis has also been used as a means of control in the evaluation of fluorometric methods (Dye 1963, All 1964b, Cum 1964, Wat 1966).

A neutron source of high intensity, e.g. a reactor, is, however, necessary for a sufficient activation of nanogram amounts of selenium, and therefore the use of the method has been limited despite its advantages.

For the present work the neutron activation method was chosen, except in a single experiment where the atomic absorption method could be used. As to the general use of the radio-activation analysis in biology, see Bowen (Bow 1963b, Bow 1966) and for the physical details necessary for the understanding of nuclear reactions and techniques see Fri 1955 and Cro 1960.

2.3.1. Neutron Activation

When a material is irradiated in a flux of neutrons, nuclear reactions will occur involving all elements present. With thermal neutrons (i.e. neutrons with an average energy of 0.025 eV) the dominating reaction will be the absorption of a neutron immediately followed by the emission of gamma rays, in usual notation called the (n,γ) reaction. Simultaneously other reactions such as (n,p), (n,α) or (n,2n) may occur, but almost always with much smaller probability. The following examples may illustrate the reactions:

Se-80 (n,γ) Se-81
Br-81 (n,p) Se-81
Kr-84 (n,α) Se-81
U-235 (n,f) Se-81

The production of an isotope is proportional to the neutron flux Φ (unit $n/cm^2 \cdot$ sec), to the number of atoms of the particular isotope, A, and to the probability of the reaction, i.e. the cross section (usually quoted in barns: 1 b $= 10^{-24}$ cm^2 which is the order of the geometrical cross section of the atomic nucleus). Further the production is proportional to the irradiation period, t, if the isotope produced is stable. Normally, however, the product is radioactive, and the number of disintegrated atoms has to be subtracted. As the decay follows a first-order reaction, the amount of radioactivity, D, at the end of the irradiation (after a period, t_i) will be

$$D = \Phi \cdot A \cdot \sigma \left(1 - \exp\left(-\frac{0.693 t}{t_{1/2}}\right)\right),$$

where $t_{1/2}$ is the half-life of the isotope and D the number of disintegrations per unit time.

For a particular radioactive isotope the half-life and the energies and intensities of its radiation are characteristic and may be used as a tool for qualitative analysis. A gamma-emitting isotope has a spectrum with one or more peaks of distinct energies, whereas the spectrum of a β-emitter is a continuum with a characteristic maximum energy. In the electron-capture decays (EC) and in isomeric transitions (IT), characteristic X-rays (KX) are emitted. The γ-spectrum together with a check on the half-life will in most cases allow the identification of the isotope.

Selenium has six stable isotopes. The data of interest in connection with activation in a reactor with thermal neutrons are summarized in table 2.5. Of these isotopes Se-75 has been used in several cases because the long half-life allows a chemical purification before measurement of the activity (Fin 1959, Ledd 1961, McC 1961, Koc 1962, Bru 1967, Sam 1967, Stei 1967). On the other hand a long irradiation time is necessary to obtain a good sensitivity. In biological investigations, Se-77m ($t_{1/2}$ = 17.5 sec) has been used by some

Table 2.5

Physical properties of selenium isotopes (Koc 1960, Bow 1963a, Lede 1967)

Stable isotope	Isotopic abundance %	Cross section barns	(n,γ) product nuclei	Half-life	Type of decay	Major radiations		Sensetivity*) μg	Spec.act. after irradiation for one half-life, $\Phi = 10^{14}$ n/cm^2·sec, mC/g Se
						Max. energy of β^-, MeV	KX and γ energy in MeV		
Se-74	0.87	26 ± 6	75	121 days	EC		KX, 0.121, 0.136, 0.265, 0.280, 0.401	$6 \cdot 10^{-4}$	2500
Se-76	9.02	7 ± 3	77 m	17.5 sec	IT		KX, 0.161	$3 \cdot 10^{-5}$	9700
Se-78	23.52	0.36	79 m	3.91 min	IT		KX, 0.096		290
		0.05	79	$6.5 \cdot 10^4$ yrs	β^-	0.16	No γ	2000	
Se-80	49.82	0.03 ± 0.01	81 m	56.8 min	IT		KX, 0.103	$1 \cdot 10^{-3}$	150
		0.5 ± 0.1	81	18.2 min	β^-	1.58		$9 \cdot 10^{-5}$	2500
Se-82	9.19	0.05 ± 0.025	83 m	70 sec	β^-	3.8	0.35, 0.65, 1.01, 2.02	$4 \cdot 10^{-3}$**)	46
		0.004 ± 0.002	83	25 min	β^-	1.8	0.22, 0.36, 1.88, 2.29	$6 \cdot 10^{-2}$	4

*) Defined as the mass that yields 1000 dpm after irradiation in a flux of 10^{14} n/cm^2·sec to saturation or for a maximum period of 30 days.

**) Se-82 (n, γ) Se-83, 83m $\xrightarrow{\beta^-}$ Br-83.

experimenters for the determination of selenium in biological samples (Oka 1960, Gib 1962, Gui 1963, Tom 1965, Duf 1967, Max 1967). Here a chemical separation is next to impossible because of the short half-life; hence the method must be purely instrumental: After the Se-77m has decayed, one remeasures the sample to obtain the sample background. In a few laboratories Se-81 has been used (Bow 1963, Dah 1965). The half-life allows a fast chemical separation before the measurement of the β-rays. Se-79m has been utilized in a single case (Kur 1962). But this and the other isotopes are of no great interest owing to the low sensitivities. The advantages of the three short-lived isotopes are the short irradiation times and the fast obtainment of the result. The convenience and reliability, characteristic of work with reasonably long-lived radioactivity, easily outweigh the increased sensitivity one could gain by working with, say, Se-77m. Except for experiments where a rapid result is necessary, the isotope Se-75 is the best choice.

In the activation method, interference from other elements may occur. Besides the formation through the neutron capture in selenium, some of the selenium isotopes may be formed in other reactions. The cases to be considered are Kr-78(n,α)Se-75, Br-81(n,p)Se-81, Kr-84(n,α)Se-81, and Se-81 from fission of U or Pu. But owing to their small probabilities all these reactions will only be of importance when the interfering element is present in a much higher concentration than the selenium itself. Therefore Br-81 (n,p)Se-81 is the only reaction that may introduce an error when biological materials are analysed. However, with a bromine concentration of 10 ppm and a selenium concentration of 0.01 ppm and irradiation with fast neutrons, the error should not exceed 2 % (Bow 1963), with a purely thermal spectrum the reaction does not take place. In soil an error of 5 % from fission in uranium can be calculated for a sample containing twice as much uranium as selenium (the average for shales). The fission yield of Se-81 is 0.13 %. Errors much above this should be rare.

The irradiation facilities in the Danish reactor DR2 are seen in fig. 2.1. The R-tubes are intended for irradiations of not more than a few hours. These tubes have pneumatic in- and output of the samples. For safety reasons the six S-tubes cannot be used for input and output of samples during the reactor run. In the R- and S-tubes containers of about 30 ml volume are used, and each tube can accomodate only one container. The V-tubes offer the highest neutron flux, but they are for smaller samples only (volume less than 17 ml), and furthermore the flux gradients are high in these positions, which makes them unsuitable for activation analysis (difference in dose to sample and reference is possible). In the thermal column all neutrons are slowed down by the graphite moderator, and the gamma-rays are absorbed so that the flux is purely thermal. In the thermal column graphite stringers are placed with sites for several containers of various sizes. For the present work a stringer with six holes and one with 24 holes were used. The 6-hole stringer receives a flux

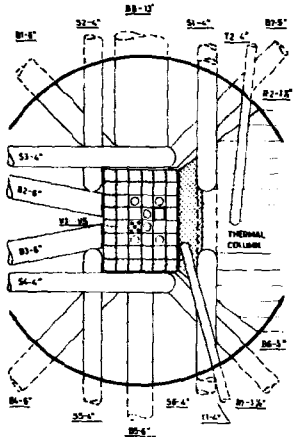

Fig. 2.1. The reactor DR2. Schematic drawing showing positions of experimental tubes and thermal column relative to the reactor core. For activation purposes the S, R and V tubes and the thermal column can be used. In the S tubes thermal fluxes from $0.5 \cdot 10^{13}$ to $1 \cdot 10^{13}$ n/cm^2 · sec are available. The flux in the pneumatic tubes R1 and R2 is about $8 \cdot 10^{12}$ n/cm^2 · sec, and in the thermal column it varies from 10^{11} to 10^{13}. The narrow V-tubes offer fluxes from $0.5 \cdot 10^{14}$ to $1 \cdot 10^{14}$ n/cm^2 · sec.

of $2 \cdot 10^{12}$ n/cm^2 · sec, while the flux in the 24-hole stringer decreases with the distance from the core from $6 \cdot 10^{12}$ to $1.6 \cdot 10^{12}$ n/cm^2 · sec. The holes are sized for 30 ml containers and placed two together. For the flux gradients to be eliminated, the 24-hole stringer was turned in the middle of an irradiation period. In this way the difference between the highest and the lowest dose was restricted to 20 to 30 %. As the thermal column points straight into the reactor, samples cannot be taken out during the reactor run. Thus the use of this facility for the production of short-lived isotopes is excluded.

In all the tubes, but not in the thermal column, the flux of fast neutrons and γ-rays is so intense that decomposition of organic material into gases is probable. This may give rise to pressure inside the tight sample container, which may result in leakage and radioactive contamination of the cooling system in the reactor. The amount of organic material is therefore restricted to the order of milligrams in these tubes. Irradiation of larger amounts of organic material must take place in the thermal column, where the temperature rise is limited, and where there is no direct contact with the cooling water.

Organic samples cannot be dry-ashed without loss of selenium. Therefore, for such material it was possible to use only the thermal column, and consequently only Se-75 could be used in these analyses. Most of the soil samples contain less than 3 % organic matter, which means that irradiation of a few grams in the S-tubes is possible when 100 mg of organic material is the upper limit.

The reactor is only run for about six hours a day four or five days a week, which in average gives one hundred hours a month. Even in the thermal column an irradiation time of more than four weeks is not convenient, because of the delay of the result and the number of samples compared with irradiation sites. This means that with a flux of 10^{12} n/cm^2·sec, the sensitivity taken as 1000 dpm (see table 2.5) with Se-75 becomes 0.4 μg. One tenth of this, however, is still measurable, and the sample size can be ten grams or more.

2.3.2. Pretreatment of Samples

Before irradiation precautions have to be taken so that chemicals, apparatus, glass-ware, etc., do not contribute to the selenium content of the sample. This means that as few operations as possible should be performed before the irradiation. Aluminium is often used as packing material because it is relatively pure. Silica is preferred to glass because the components of glass would cause a great amount of radioactivity. Aluminium as well as silicon give only a short-lived isotope (Al-28 with $t_{1/2}$ = 2.3 min and Si-31 with $t_{1/2}$ = 2.6 hours).

To avoid pressure build-up due to the radiolysis of water, all samples must contain as little water as possible when submitted to irradiation regardless of the position in the reactor.

Activation of the selenium atoms in various compounds in the sample implies the possibility of disturbance of the chemical bond (Szilard-Chalmers reaction). Hence a chemical separation of various compounds after an irradiation will only by chance lead to the correct result. Consequently only the total content of an element can be determined in an irradiated sample. For the determination of a particular compound or fraction the isolation has to be performed prior to the irradiation.

Plants: All milled plant materials were pressed into 2-g briquettes except some materials with a high sugar content which were irradiated as powder. Grains and seeds were not ground, but irradiated as such.

Soils: The soil samples were air dried and ground in an agate mortar to pass a sieve no. 40 (ASTM) before the final sample was taken for irradiation. Later pressed soil samples of 3 g were used.

Soil extracts: One hundred grams of soil was shaken overnight with 250 ml distilled water or another extractant. The extract was filtered and evaporated in a water bath in an evacuated rotating flask. Aliquots of the aqueous

concentrate were evaporated to dryness on aluminium foil in a current of air under an infra lamp.

References: Either the exact integrated number of neutrons reaching the sample has to be known, or a known amount of selenium must be irradiated in the same position as the test sample once for all if the flux is very constant; otherwise it must be irradiated together with the test sample. Simultaneous irradiation of references and test samples was chosen because it was considered the most reliable and the simplest procedure.

For irradiation in the thermal column 5 μg of selenium as a solution of SeO_2 (100 μg Se/ml) was placed on a strip of filter paper and air dried. The paper contained less than 0.01 μg Se per strip. Four such strips were wrapped in aluminium foil and irradiated together with the test sample. Only two of them were worked up for measurement, while the other two served as a reserve.

For irradiation in the S-tubes 20 μg of selenium (400 μg Se as SeO_2/ml) was evaporated at 60°C in a 150 μl silica vessel with a stopper. Three replicates were used.

Packing: The materials were placed in cylindrical aluminium containers for the irradiation. Powder and seeds were placed directly in the containers with the references wrapped in foil. Two or three briquettes of the same sample were wrapped in foil, and two such packages were irradiated with the references placed between them.

2.3.3. Chemical Separation.

Before a measurement of the selenium activity the selenium in the sample must be separated from all other induced activities. A known amount of stable selenium - in casu 20 mg - was added as a carrier for the activated selenium, whereby work with quantities of the order of 10^{-4} mg was avoided. Consequently the conventional technique was applicable, and the known amount of carrier allowed the determination of the chemical yield. For the same reason it may be an advantage to add hold-back carriers for other elements which might follow the selenium in trace amounts and cause a low radiochemical purity. This was, however, not necessary when Se-75 was the item.

The initial activity in a sample may be uncomfortably high, and in the present work this was usually due to sodium and potassium ($t_{1/2}$ = 15 h and 12 h respectively). Thus the newly irradiated soil samples were rather "hot" in contradistinction to plant material. Hot samples were allowed to cool for about one week before the chemical separation.

Combustion: Generally dry ashing cannot be used because of the volatility of many selenium compounds. At present the methods most used are oxygen flask combustion (Dye 1963, All 1964b, Lane 1966b, Tau 1966, Wat 1966) and wet oxidation with nitric acid plus perchloric acid (Bow 1963a, Gar 1963, Lin 1965, Wat 1966).

Neither Watkinson (Wat 1966) nor Dye et al. (Dye 1963) found any loss of selenium in the oxygen combustion. Bock and Jacob (Boc 1964), however, gave attention to the possibility of a 10 % loss if the excessive oxygen was not trapped in an absorbent.

The recovery of selenium can be incomplete after wet combustion with various oxidants. Thus a loss takes place when the oxidants are nitric acid plus sulphuric acid and the temperature is increased too fast, or if charring occurs (Gor 1959, Boc 1964). According to Bock and Jacob the addition of perchloric acid to the oxidation medium decreased the loss from 15 to 9 %, but the recovery was further improved if sulphuric acid was omitted (Boc 1964). Lindberg has reported a 10 to 30 % loss of selenite-Se added to grain when the combustion with nitric plus perchloric acid was too fast (Lin 1968). According to other investigations perchloric acid prohibits any loss of selenium (Fog 1956, Gor 1959).

In the investigations cited above organic materials with added inorganic selenium were decomposed. Under these conditions it would not be possible to control any loss of organic selenium not yet oxidized. Aware of this problem, some authors (All 1964b, Wat 1966) have compared results obtained after nitric-perchloric-acid combustion, oxygen combustion and neutron activation followed by wet combustion. As far as it was possible to conclude from the material, no loss occurred during the wet combustion. Also Lindberg (Lin 1968) using Se-75-labelled animal tissues and nitric plus perchloric acid recovered 100 % of the activity.

In materials with a high sugar or starch content such as beets and grains selenium may be reduced to elemental selenium, or charring followed by loss of selenium may occur if insufficient nitric acid is present. In a few other materials, however, lack of nitric acid may even cause an explosion in the perchloric acid solution. Mustard seed for instance has this property, and in soil samples the formation of $Mg(ClO_3)_2$ is possible. Hence it might be better in such cases to use nitric acid alone or dry oxidation.

In an experiment (table 2.6) with Se-75-labelled plant material the nitric acid-perchloric acid decomposition was assessed, and in addition it was attempted to find a suitable method to be used with mustard seed and other oily samples. For this purpose wet combustion with nitric acid-perchloric acid or nitric acid alone was compared with dry combustion with sodium peroxide in a bomb or with alcoholic magnesium nitrate (Kro 1958). After the decomposition selenium was isolated and the activity compared with the original one (table 2.6). Only nitric acid plus perchloric acid gave a satisfactory destruction. As would be expected, the decomposition with nitric acid alone is only partial; for these materials 30 to 50 % of the selenium was released. For sodium peroxide fusion it was possible to use only 0.25 g samples, and this may be the reason for widely varying results, especially with mustard seed. This method was therefore ruled out. The

Table 2.6

Comparison of methods for destruction of plant material. Plants labelled with Se-75 were used. After combustion Se was isolated. The results are expressed in per cent of the original activity. The numbers in parenthesis are the numbers of plant samples. Each analysis was made in duplicate.

Plant species	Combustion			
	HNO_3+$HClO_4$ (2:1)	HNO_3	Na_2O_2 in bomb	Alcoh. $Mg(NO_3)_2$
Rye grass, red clover	102 (5)	54 (2)	110 (2)	72 (3)
Barley, green	107 (2)	38 (2)		81 (2)
Barley and mustard straw	103 (1)	46 (3)		
Radish top	102 (3)			
Mustard, green	102 (5)	32 (2)	101 (2)	73 (1)
— , seed		51 (6)	116 (2)	59 (3)

recoveries after the magnesium nitrate ashing correspond well with the recovery of sulphur after the same ashing (see table 2.14). Though the alcoholic magnesium nitrate gave better recovery than nitric acid, the latter was preferred for mustard seed and other oily materials because magnesium nitrate ashing with radioactive materials could cause contamination. But as seen, only about half the selenium in the seeds will be released, and the presence of nitric acid excludes separation by distillation.

According to Rosenfeld and Beath (Ros 1964), soil selenium will in most cases be released by distillation without any pretreatment. For some rocks the authors recommend oxidation with nitric-sulphuric acid. However, with this mixture a selenium loss could be expected, and if perchloric acid was added, a risk of detonation was introduced. Consequently hydrogen peroxide was used instead as it gave sufficient decomposition and oxidation for complete recovery of the selenium (subsection 2.3.6). No loss of added Se-75 selenite was found.

To sum up: plant materials except oily seeds were combusted with a mixture of 16 N HNO_3 and 70 % $HClO_4$ in a ratio of 2:1 or higher, while oily seeds were combusted with nitric acid alone. Soils were boiled with 30 % hydrogen peroxide. The sample size was from 0.5 to 4 grams depending on the material and the expected selenium level. Aluminium foil with the residue from evaporated soil extracts was dissolved in hydrochloric acid. The evaporated extract corresponded to about 25 g soil.

Isolation by Distillation: When Se^{+4} is treated with strong hydrobromic acid, volatile $SeBr_4$, which can be distilled off, is formed. Nitric acid prevents the reaction, and it is therefore important that all the nitric acid from the wet combustion has been driven off. The procedure and the apparatus for the distillation closely resembled that described by Leddicotte (Ledd 1961). A

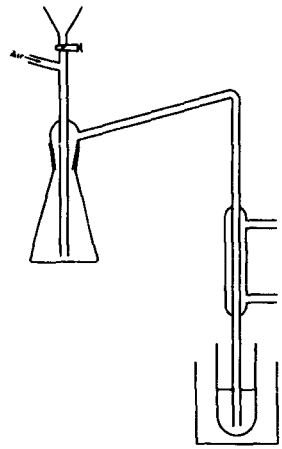

Fig. 2.2. Apparatus for selenium distillation. A 150 ml conical flask with a ground joint is provided with a unit comprising an air-inlet tube and a connection to a condenser. The distillate trap is placed in an ice-bath. (After Ledd 1961)

funnel was added to the apparatus (fig. 2.2) because it is sometimes necessary to add more hydrobromic acid or to add bromine if the selenium compounds have remained in the condenser. Six such apparatus units could be placed in the fume hood.

Twenty mg Se as SeO_2 was added to a known amount of the irradiated sample. The sample was digested in a conical flask fitting the distillation set-up. Twenty ml of concentrated hydrochloric acid (for the reduction of Se^{+6} to Se^{+4}) and 20 ml of 48 % hydrobromic acid were added to the digested material. The application of hold-back carriers was found unnecessary when Se-75 was used, while the radiochemical purity of Se-81 had been improved by the addition of arsenic and other hold-back carriers. The distillate was collected in a 100 ml centrifuge tube placed in an ice bath and containing 20 ml of water and 10 ml of concentrated hydrochloric acid. For the distillation to be accelerated a current of nitrogen or air had to be passed through the system. Air was used because the chemical yield was independent of the gas. After a few minutes of heating the redbrown selenium tetrabromide passes the condenser, and the distillation can be stopped shortly after. The distillate was neutralized with concentrated ammonia (the bromine colour disappears), and 2 ml conc. HCl was added to ensure that selenium was present in the +4 state. Further 0.5 ml of a 1 % aerosol solution was added to prevent the selenium precipitate from adhering

later on to the glass surface. For the precipitation of elemental selenium Leddicotte (Ledd 1961) used sulphurous acid, and Bowen and Cawse (Bow 1963a) used gazeous sulphur dioxide. It was, however, found more practical to use 0.8 to 1.0 g hydrazine sulphate for the reduction. The process was accelerated by heating in a boiling-water bath for half an hour. For this purpose crystallization cups with a diameter of 15 cm and a depth of 7 cm were used. They were covered with perspex lids with holes for seven centrifuge tubes. The decantation of the solution without loss of selenium is impeded by the presence of solid NH_4Cl, and therefore the tubes were centrifuged while still hot to avoid precipitation of NH_4Cl from the nearly saturated solution. The selenium precipitate was washed twice with water and once with ethyl alcohol and transferred to a weighed aluminium cup with a diameter of 30 mm and a depth of 8 mm by means of a little alcohol. The alcohol was evaporated under an infra lamp in a current of air, and the yield was determined. The selenium was fixed in the cups with a few drops of a cellulose lacquer diluted with acetone.

Table 2.7

Influence of the material on the chemical yield of selenium isolated by distillation

Material	Yield, %
Cereals, grain	67 ± 13
— , straw	70 ± 12
Pasture species	70 ± 15
Beets, roots	63 ± 15
Soils	67 ± 12
Soil extracts	60 ± 12
References	95 ± 3

The chemical yield varied somewhat with the material as seen from table 2.7, but generally it was between 60 and 70 %. The yield of the references was 95 %. This difference is, of course, of no importance as long as the chemical exchange can be assumed to be complete.

Dithizone Extraction: After digestion with nitric acid alone another method than distillation as $SeBr_4$ was needed for the isolation of selenium. It was found that selenium is reducible from the neutralized nitric acid digest, and that it precipitates partly just by standing. The selenium precipitate, however, was very impure. But if the selenium was reprecipitated twice, the final product was chemically pure (constant specific activity), though contaminated with a few other radioactive isotopes. Hg-203 ($t_{1/2}$ = 47 days, γ-ray 279 keV) was the most serious one owing to the γ-ray energy very close to one of the Se-75 γ-rays (subsection 2.3.5). Other impurities had γ-energies

above 500 keV, and hence small contaminations would not disturb the measurement of Se-75 much. To overcome the disturbing comtamination an extraction with dithizone was inserted before the reduction.

Mercury forms a complex with dithizone; at a pH up to 4 it is in the form of the acid dithizonate. Both dithizonates are extractable with chloroform and carbon tetrachloride (Iwa 1958). In a tracer experiment Hg-203 and Se-75 were added to a nitric acid digest, and the extraction of Hg - and of Se - with dithizone in chloroform was followed at different pH-values. At a pH between 1 and 2 about 80 % of the mercury was extracted into the organic phase, while only about 1 % of the selenium was extracted.

Hence the combusted sample was filtered, neutralized with 10 N NaOH to a pH between 1 and 2 and immediately afterwards shaken for one minute with 10 ml 0.1 % dithizone in chloroform. If the green colour of the organic phase disappeared, the extraction was repeated. A rather large excess of dithizone is needed as the dithizone is destroyed by nitric acid. The aqueous phase was transferred to a centrifuge tube, and 2 ml conc. HCl, aerosol and 1 g hydrazine sulphate were added for reduction to the elemental form. The selenium was washed with water and alcohol, and the tubes were placed in a cupboard at 80°C until dry. The solid was dissolved in a few drops of nitric acid while still hot, water was added, and the precipitation repeated twice, now with 0.8 g of hydrazine sulphate, and the selenium was mounted for counting. Besides a reduction of the mercury contamination by a factor of five, an important part of the unidentified impurities was removed.

References: References on filter paper were placed in test tubes containing 0.1 mg selenium carrier. One ml HNO_3 : $HClO_4$ (1:1) was added, and the test tubes were kept in a boiling-water bath until the paper was destroyed. The solution was diluted and transferred to a centrifuge tube containing 20 mg selenium. Two ml conc. HCl and 0.5 ml 1 % aerosol solution were added, and selenium was precipitated once with 0.8 g hydrazine sulphate.

During irradiation some of the selenium can be reduced to the ground state. For this reason references in silica tubes and their stoppers were left overnight in small beakers with 20 mg selenium carrier and enough nitric acid to cover the tubes. The following day the solutions were boiled for a few minutes to ensure complete dissolution of the selenium. The solutions were transferred to centrifuge tubes, the tubes were washed with water, and the selenium was precipitated and mounted as above.

2.3.4. Counting Technique

Gamma rays have three main types of interaction with matter: (1) photo-electric effect, (2) Compton scattering and (3) pair production. The first process is the most important for low gamma energies. By this process the entire photon energy is given to a bound electron, and the probability of

photoelectric absorption is thus connected with the binding energies of the K and L electrons. The result is distinct peaks in the gamma spectrum (number of events versus energy) as measured with a scintillator or solid-state counter. By the second process part of the photon energy is transferred to a bound or a free electron, and the photon is scattered. The third process can only occur when the photon energy is above 1022 keV, and the probability is proportional to Z^2. The pair production results in a 511 keV peak plus a peak at $(E_\gamma - 2 \cdot 511)$ keV. All three processes are favoured by a high atomic number of the absorbing material.

The most frequently used detection method for gamma rays is the scintillator method. Of most general use is the sodium iodide crystal activated with 0.1 % Tll. Sodium iodide crystals are produced in sizes up to several inches in each dimension. For γ-spectroscopy, crystal plus photomultiplier sets specially selected for high-energy resolution are available. (The resolution is defined as the relative width of a gamma peak at half the maximum count rate. The standard reference line is normally taken as the 662 keV peak of Cs-137.)

In the years since 1963, solid-state detectors for gamma counting have been developed. Among these, germanium crystals drifted with lithium have proved advantageous for many purposes compared with NaI-crystals, because of their much better energy resolution. The size of the germanium crystals available is still being increased, and volumes of 30 cm^3 can be obtained. As the energy gap for electron-hole pair production is very small, it is necessary to cool the germanium detectors to −170°C to reduce the noise level.

For the activation analyses a NaI-detector was used, but for comparison a few measurements were made with a germanium counter*.

Scintillation Counting. A 3 x 3" cylindrical NaI-crystal was used. Detector and photomultiplier were shielded by 10 cm of lead (fig. 2.3). The radioactive sample was placed in a source holder on the top of the detector (fig. 2.4). The holder was provided with notches making it possible to place the disk with the source at three different distances from the detector.

The detector was connected with a multichannel analyser (Nuclear Data Model 130A) with 512 channels (figs. 2.5 and 2.6). In the analyser the signals are amplified and registered in the proper channel according to the energy. An approximate linear relationship exists between channel number and energy. The calibration can be carried out with a few gamma emitters with characteristic gamma rays, such as (fig. 2.7) Hg-203 (77 and 279 keV), Cs-137 (662 keV), Co-60 (1170 and 1330 keV).

The gamma spectrum of Se-75 is shown in fig. 2.8. The area below the first peak or the first two peaks was used for calculation of the amount of selenium, but all three peaks were used for control of the radiochemical

* I am indebted to Mr. K. Wilsky, formerly of the Chemistry Dept., Risö, who placed the detector at my disposal.

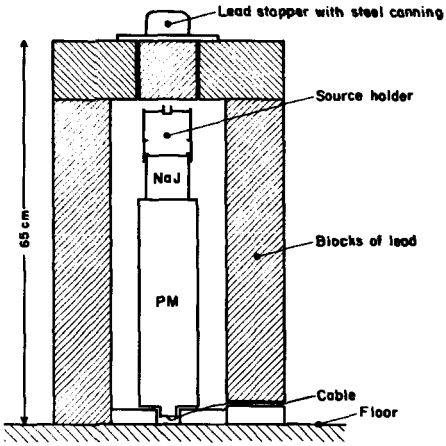

Fig. 2.3. Lead shielding of the 3 x 3" NaI-crystal and the photomultiplier (PM). The connection to the analyser is taken out from the bottom.

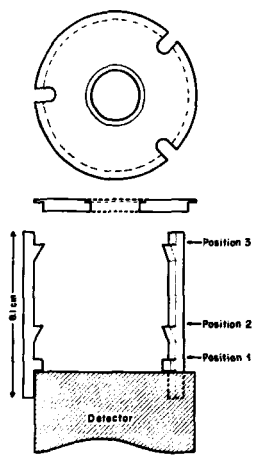

Fig. 2.4. Sample holder in perspex. The holder fits on top of the detector. A disk with a hole for the counting cup can be placed at three distances from the detector.

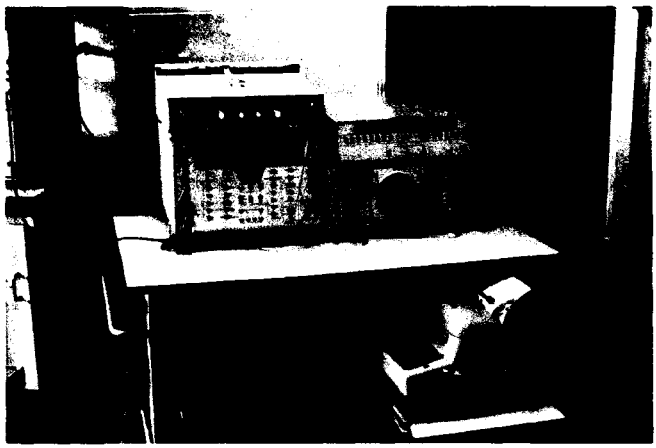

Fig. 2.5. The counting equipment. From the top of the rig the units are: Current supply, X-Y writer, oscilioscope, 512 channel analyser, calculation unit, and high-voltage supply. To the right of the rig is the lead house with the detector.

purity. If the ratio between the areas differed from the corresponding ratio found by means of the reference sample, that was an indication of an impurity. Of course most impurities would be observed simply by inspection of the display on the oscilloscope. But for routine work and especially if the impurity was Hg-203 (279 keV), the presence of the impurity may be overlooked because it merges with the central peak in the selenium spectrum (fig. 2.8).

Mostly position 1 in the sample holder (fig. 2.4) was used. The counting efficiency in this position is about 40 % for Se-75. In cases of a total count rate of more than 60,000 cpm (counts per minute) the sample was placed in position 2 or 3 to avoid dead-time losses and drift of the amplification in the photomultiplier tube. Count rates for Se-75 in the three positions are shown in table 2.8.

With a 3″ crystal the background was about 1 cps. After irradiation in a flux of $4 \cdot 10^{12}$ n/cm^2 · sec for 100 hours we can detect $1.5 \cdot 10^{-3}$ μg Se or 0.0003 ppm in a 5-g sample. For a quantitative determination about 0.0015 ppm is necessary. At low count rates the areas below both peak 136 and peak 265 are used, which increases the statistics of the counting. The weaker peak 401 should not be included because it would decrease the signal to noise ratio a little.

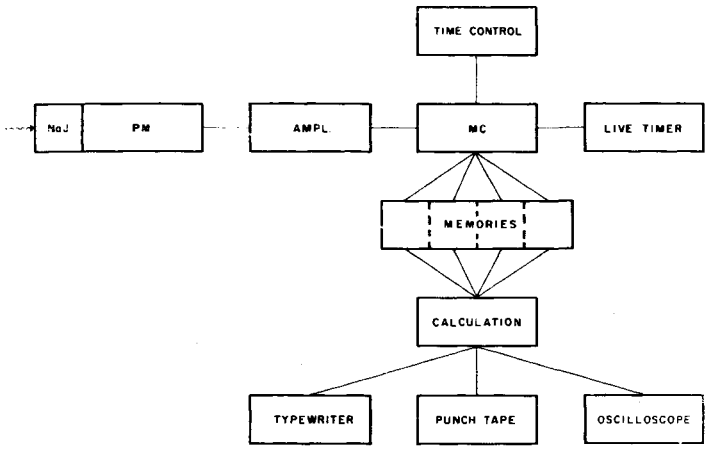

Fig. 2.6. Block diagram of the scintillation counting equipment. The pulses are amplified before entering the pulse height analyser (MC). The time control presets the counting time, and the live-timer corrects for the dead time in the analyser at high counting rates. The information is stored in 128, 256 or all 512 channels of the memory. From there the information is transferred to an oscilloscope, to a punch recorder, to a typewriter or to a X-Y writer. The time for typewriting of 512 figures is about seven minutes, for punching only two minutes.

One or two of the four memories can be used for storage of a background or standard spectrum which by means of the calculation unit can be subtracted from (or added to) a spectrum in another memory, and the spectra can be multiplied by factors of ten. This makes it possible to subtract a background from the measurement even when the two measurements have been made for a different period of time. Another option is summation of a spectrum or part of it. This can be done inside a 128 or 256 channel memory, but not in all 512 channels because the first 256 channels act as receptors during the calculation.

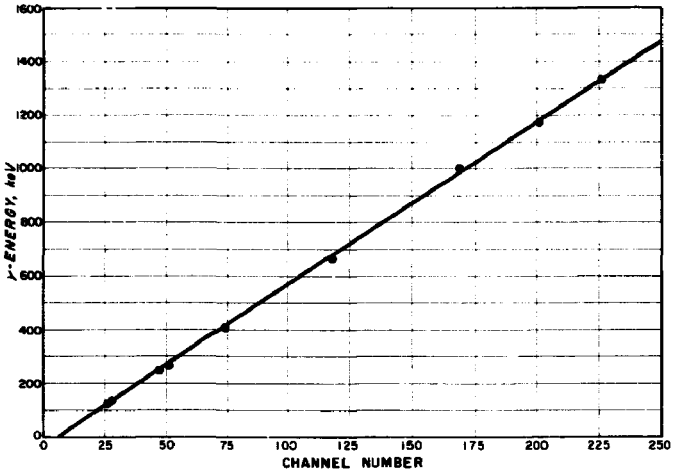

Fig. 2.7. Energy calibration curve. The isotopes used here are Eu-152, Se-75, Cs-137, and Co-60.

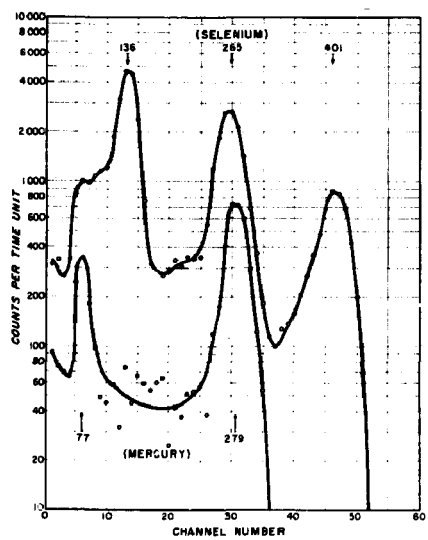

Fig. 2.8. Gamma spectra of Se-75 and (below) Hg-203 measured with a 3 x 3" NaI-crystal. The geometry was close to 50 %. The energies of the most important gamma rays are shown.

Table 2.8

Count rates in the three positions of the sample holder

Position no.	Distance from detector, mm	Counts per min		
		136 keV peak	265 keV peak	401 keV peak*)
1	~2	10,000	7,400	3,600
2	17	6,900	4,800	1,300
3	60	2,500	1,700	300

*) The count rate in position 3 is only 9 % of that in position 1 for this γ-ray (as compared with 25 and 23 % for the two others). The reason is that 64 % of the events with the energy 401 keV are a sum line (136 keV + 265 keV).

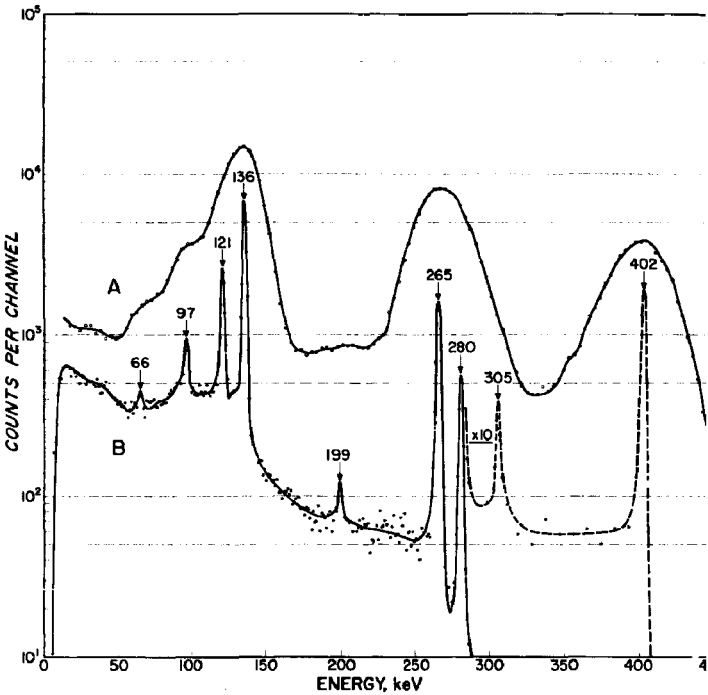

Fig. 2.9. Spectrum of Se-75 measured with a 6 cm² x 5 mm Ge-Li detector in connection with a 1024 channel analyser (curve B). (The crystal was made by J. Lippert, The Health Physics Dept., Risø, early in 1966). Curve A is the selenium spectrum measured with a 3 x 3" NaI-crystal. The counting efficiency of the Ge-counter is about 20 % of that of the NaI-crystal. One channel corresponds to about 0.5 keV

Semi-conductor Counting. The difference between a NaI- and a Ge-Li crystal with respect to resolution is illustrated in fig. 2.9. With the germanium counter both the 136- and the 265-peaks are resolved into two peaks. The high resolution means that the germanium counter offers new possibilities for the activation analysis as a purely instrumental analysis or at least with less chemical isolation than now. Further it will be easier to determine more than one element in the same sample. This is becoming possible as the size of the crystals increases.

2.3.5. Calculation

The numbers of counts for one gram of test material and for 1 μg of reference selenium are calculated by means of the chemical yield, the amount irradiated and the count rate below one or two peaks. Selenium in the test is then obtained from the proportionals. The whole calculation can be written as

$$\text{ppm Se} = \frac{c_T \cdot b_T}{W_T \cdot a_T} \cdot \frac{W_R \cdot a_R}{c_R \cdot b_R}$$

where a_T is grams of test sample and a_R is μg of Se-reference irradiated, b_T and b_R are the carrier amounts in mg, c_T and c_R are the count rates in cpm and corrected for background, and W_T and W_R are the chemical yields in mg.

Table 2.9

Standard deviation of the selenium analysis made by neutron activation.

Se content	Replicates	Standard deviation in per cent
5 or 20 μg (references)	2	1.05
0.01-0.05 ppm	3	5.9
0.05-0.5 ppm	3	5.5
0.5-50 ppm	3	6.4

The standard deviation of the averaged results was calculated for three concentration intervals. The calculation was in each group based on forty analyses. The test material in each group comprised barley grain, red clover, beets, and mustard straw. The test samples were analysed in triplicate (2 g each), the reference was in duplicate (table 2.9). Regardless of the concentration the standard deviation is about 6 % for the test specimens and 1 % for the reference.

2.3.6. Comparison of Results Obtained by Neutron Activation and Fluorometric Determination

As a control of the procedure three samples already assayed by a fluorometric method were analysed (table 2.10). The agreement was very satisfactory for the soil samples, whereas for kale the activation analysis yielded a somewhat higher result than the fluorometric determination, but the difference is not significant.

Table 2.10

Comparison of results by neutron activation and fluorometry.

Sample*)	ppm Se	
	Fluorometric determination**)	Neutron activation
Soil No. 357	35.5	36.0
Soil No. 370A	21.6	22.5
Standard kale	0.137	0.155

*) The samples were kindly supplied by Dr. G. Fleming, The Agricultural Institute, Wexford, Ireland.
**) Analysed by Dr. J. C. Lane (Lane 1966b).

2.3.7. Determination of Se by Atomic Absorption

This method is mostly used for the determination of metals, but also metalloids can be determined, although with much less sensitivity. The method is based on the following principle: The test solution is sprayed into a flame and atomized. Simultaneously, light of one of the characteristic spectral wave-lengths of the element to be analysed is directed through the flame. The light source is a hollow cathode lamp. The intensity of the beam is measured, and the amount absorbed by the atoms is proportional to the concentration of the element in the solution.

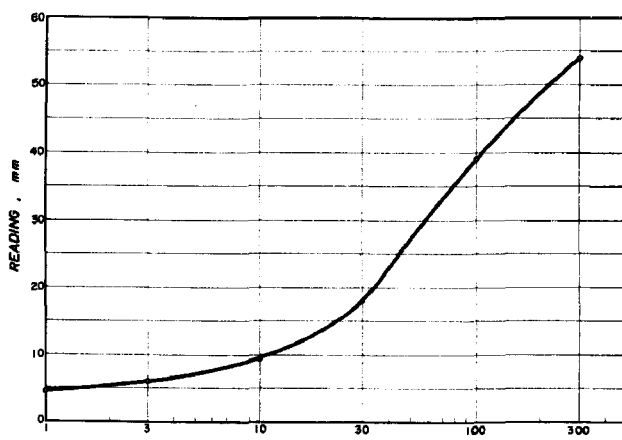

Fig. 2.10. Selenium measured by atomic absorption. Standard curve of Se in 14 % $HClO_4$ (v/v). Jarrell-Ash apparatus, 196 mμ cathode lamps.

According to the Perkin-Elmer manual the sensitivity for selenium is about 2 ppm in the final solution, and the limit of detection is 1 ppm. Thus only highly seleniferous samples can be analysed by this method, and for that reason the method was only used in two experiments (pot experiments Nos. 7 and 8). At our disposal was a Jarrell-Ash atomic absorption/flame emission apparatus and a cathode lamp emitting light of 196 mμ intended for selenium determination. The organic material (0.5 g) was decomposed with HNO_3 + $HClO_4$; the mixture was diluted with water and filtered through glass wool. The final volume was 25 ml and the concentration about 14 % (v/v) with respect to $HClO_4$. The calibration curve is given in fig. 2.10. As it was only the aim to obtain an approximate Se concentration in the material in question, no attempt was made to optimize the working conditions. The limit of detection was under our conditions 250 ppm in the dry matter.

2.4 Analytical Techniques in Tracer Experiments

By means of Se-75 the plant uptake of different selenium compounds was followed in pot experiments. In this connection a rough characterization of the selenium compounds formed in the plants was desirable. In the soils it was of interest to follow a change in oxidation state and in solubility of the added selenium salts. In another series of experiments the influence of selenium on the sulphur uptake was followed, and here a characterization of the plant sulphur was required.

2.4.1. Oxidation States of Selenium in an Aqueous Extract (Procedure I)

Beath and Eppson (Bea 1947) used a water extract of the soil or the plant and separation of the dissolved selenium into selenate, selenite and organic selenium. According to these authors a suitable extract of a soil is obtained by overnight shaking of 200 g soil with 500 ml water. Alternative suggestions are refluxing for thirty minutes or heating over a steam bath for several hours followed by standing overnight. Plant materials are extracted with boiling water (500 ml to 50 g dry sample). The total amount of water-soluble selenium is determined by digestion of the extract followed by distillation. Selenite plus selenate are determined by precipitation with SO_2 and hydroxylamine after the addition of one volume 48 % HBr. It is here assumed that the organic selenium is not co-precipitated. Finally the selenite is precipitated from another aliquot either with SO_2 after addition of H_2SO_4 or with ascorbic acid.

The extraction of soil by shaking overnight was compared with treatment in a water bath for various lengths of time. Selenite or selenate had been added to two soils two months previously, and the soils had been stored with a moisture content of about 15 %. Water and solutions of potassium selenite or selenate containing 50 ppm Se were used as extractants. The results for the two soils (soil No. 2, a loamy sand and soil No. 5, a sandy clay loam)

Table 2.11

Extraction of selenium from a sandy clay loam (soil No. 5) two months after the addition of 0.1 ppm Se as K_2SeO_3 or K_2SeO_4. The results are expressed in % of the added Se.

Conditions	Soil: soln. ratio	Se added as	Extractant		
			Water	50 ppm Se^{+6}	50 ppm Se^{+4}
Water bath, 3 h	1:12	Se^{+6}	32	32	40
Shaking overnight	1: 2.5	-	18	19	25
Shaking overnight	1:16	-	18		
Water bath, 3 h	1:12	Se^{+4}	38	40	60
Shaking overnight	1: 2.5	-	15	17	28
Shaking overnight	1:16	-	19		

were similar, and those for the latter are shown in table 2.11. For details about the soils, see table 3.2. Independently of the soil:solution ratio, shaking at room temperature was less efficient than extraction at higher temperatures. It is seen from fig. 2.11 that after about one hour of extraction in a water bath the extraction was completed. Nevertheless, an extraction time of three hours was chosen as a precaution. As a rule a selenite solution

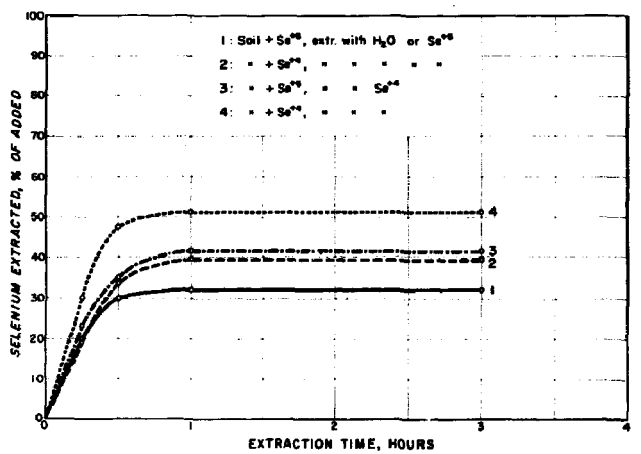

Fig. 2.11. Extraction of selenium from a sandy clay loam (soil No. 5). Extraction in a water bath. Soil:solution = 1:12. The selenium was added two months before as either K_2SeO_4 (Se^{+6}) or K_2SeO_3 (Se^{+4}) in an amount corresponding to 0.1 ppm Se.

extracted more selenium than did water or a selenate solution. From the results of the fractionations (see later) it will appear that the excess is a contribution from the exchangeable selenite.

Other extractants have been tried in preliminary experiments. These extractants were diluted acetic acid and hydrochloric acid, ammonium acetate and sulphate and calcium chloride. Only ammonium acetate extracted the same amount of selenium or more from the soils than did water, but the differences between the extractants were small.

If the extraction of the soil with water is followed by an extraction with 6N H_2SO_4, the remaining selenium should be dissolved (Will 1936). The presumption was that the selenium is present in ferruginous compounds. In the present series of experiments, only part of the selenium was dissolved in H_2SO_4 (see figs. 5.1 and 5.2), and it would seem that the characterization of this fraction is still an open question.

For plant material it was found that twenty parts of water was a more adequate amount than the ten parts suggested (Bea 1947). Extraction times from 1/4 to 3 hours in the water bath at about 80°C were tried. No difference was found in the total amount of extractable selenium, and half an hour was chosen.

The selective precipitation of selenite was tested by means of Se-75 in the oxidation states +4 and +6. Besides the procedure described by Beath and Eppson (Bea 1947), a precipitation with stannous ions has been suggested by Buketov et al. (Buk 1964). They used 300 to 400 mg $SnCl_2$ and a reaction time of two hours for the precipitation of 10 mg selenite-selenium in 25 to 50 ml 5 % HCl in the presence of selenate. In a preliminary experiment stannous chloride was found a more promising precipitant than ascorbic acid (Bea 1947).

Precipitation with increasing amounts of stannous chloride was therefore tried on solutions containing 9 mg Se as selenate and 10 mg Se as selenite. Mostly the latter was labelled with Se-75. Both 6N H_2SO_4 and 5 % HCl were used as media. From table 2.12 it is seen that the amount of $SnCl_2$ is much more critical in hydrochloric acid than in sulphuric acid, and that the necessary amount is not quite in agreement with that mentioned by Buketov et al. The selenite was precipitated quantitatively in both media with 100 mg $SnCl_2 \cdot 2H_2O$ as seen from the count rates. In the hydrochloric acid medium, however, a coprecipitation of the selenate took place, and with 200 and 400 mg stannous chloride the formation of the red selenium precipitate continued for more than one hour. In the sulphuric acid medium no precipitation of selenate took place as seen from the use of labelled selenate (table 2.12). Though the yield with 400 mg $SnCl_2 \cdot 2H_2O$ was 12.8 mg, the count rate only corresponded to about 0.3 per cent of the added activity or 30 μg Se. Therefore the increase in yield with 400 mg $SnCl_2 \cdot 2H_2O$ in the

Table 2.12

Reduction of 10 mg selenite by stanno chloride in the presence of 9 mg selenate. Reaction time: 15 minutes at room temperature, total volume: 20 ml. The amount of Se-75 used corresponded to 10,400 c/10s measured on the precipitate.

$SnCl_2.2H_2O$	Reduction in 6 N H_2SO_4				Reduction in 5 % HCl	
	Se^{+4} labelled		Se^{+6} labelled*)		Se^{+4} labelled	
mg	mg Se	c/10 s	mg Se	c/10 s	mg Se	c/10 s
50	8.8	8,200			8.0	8,600
75	10.4	10,100				
100	10.1	10,000	9.6	5	13.7	10,400
150	10.1	10,250				
200	10.6	9,900			16.3**)	10,600
400	13.1	10,000	12.8	30	16.2**)	10,300

*) Same amount of activity as used for Se^{+4}

**) Red precipitate formed in the filtrate

Table 2.13

Reduction of 10 mg selenite in the presence of 9 mg selenate and soil or plant extracts. Reduction with 100 mg $SnCl_2 \cdot 2H_2O$ in 6 N H_2SO_4 and a reaction time of 15 min.

Extract of		Volume before reduction, ml	Se^{+4} labelled			Se^{+6} labelled		
material	g		mg Se	c/10 s	counts in % of added Se-75	mg Se	c/10 s	counts in % of added Se-75
Soil	0.25	20	10.5	19,900	103			
	0.5	20	10.8	28,970	98	10.5	90	0.5
	0.5	60	10.4	19,100	99			
Beet (root)	0.1	20	10.9	18,700	97	11.1	145	0.7
	0.25	20	11.2	18,320	94			
	0.25	60	10.8	18,900	98			
	0.5	20	10.7	15,120	78			
Clover	0.05	20	10.5	18,600	96	12.0	860	4.4
	0.15	60	10.6	18,500	96			
	0.25	20	11.6	16,770	87			
	0.5	20	11.3	13,840	72			
Water control		20	10.3	19,670	100	10.3	49	0.25
		60	10.3	19,000				

sulphuric acid medium must have been due to another precipitate, perhaps SnSe. The reaction time was varied from five minutes to half an hour. It was found that five minutes was too little, but that fifteen minutes was adequate.

The selective reduction with 100 mg $SnCl_2 \cdot 2H_2O$ of 10 mg Se as selenite in 6N H_2SO_4 was then tried on soil and plant extracts of various concentrations (table 2.13). The reduction was greatly dependent on the concentration and the source of the extracts. This means that the procedure has to be checked for each new material. Within the limits used here it is permissible to increase the amount of extract when the volume is increased correspondingly. The poorest separation was obtained in the clover extract, but for the present purpose the use of the weakest concentrations is still acceptable despite a 4 % selenate co-reduction.

Final Procedure I. Six g moist soil was subjected to extraction for three hours in a water bath with 80 ml of distilled water or a 50 ppm selenite solution. During the extraction period the samples were stirred occasionally. When cooled, the samples were centrifuged and filtered through a fast, tight filter. The residue was washed with water and the volume made up to 100 ml. If the filtrate was not clear, it was refiltered. In certain cases the extracted soil was then treated twice with 20 ml 6N hot H_2SO_4. The extracts were filtered through glass wool and made to volume. The water content in the soil was determined on another sample.

One gram of dried plant material was treated with 20 or 30 ml of water (or more if the material formed a gelatinous mass as for instance radish does). The extract was filtered and the volume made up to 50 ml.

The total extractable selenium was determined in a 10 ml aliquot by counting in a well crystal. For the plant material the percentage of soluble selenium was calculated from the previously determined total content. For soils from pot experiments a rough estimate of the same percentage was made by comparison between the activities in extracted and unextracted samples.

To obtain the selenium-fractions in the water extract we added 10 mg Se as both selenite and selenate as carriers to 10 or 15 ml aliquots. From one aliquot selenate and selenite were precipitated with 1 g hydrazine sulphate after the addition of 1 volume 48 % HBr and aerosol. After centrifugation 10 ml was counted. This count rate corrected for dilution gives the amount of organic selenium.

The selenite was precipitated from a solution containing a suitable amount of extract (see table 2.13), 10 mg Se^{+4} carrier and 10 mg Se^{+6} carrier. The volume was doubled with 12N H_2SO_4, and immediately afterwards 1 ml 10 % $SnCl_2 \cdot 2H_2O$ was added. After fifteen min at room temperature the sample was centrifuged, and 10 ml was taken for counting. The supernatant contained selenate plus organic selenium.

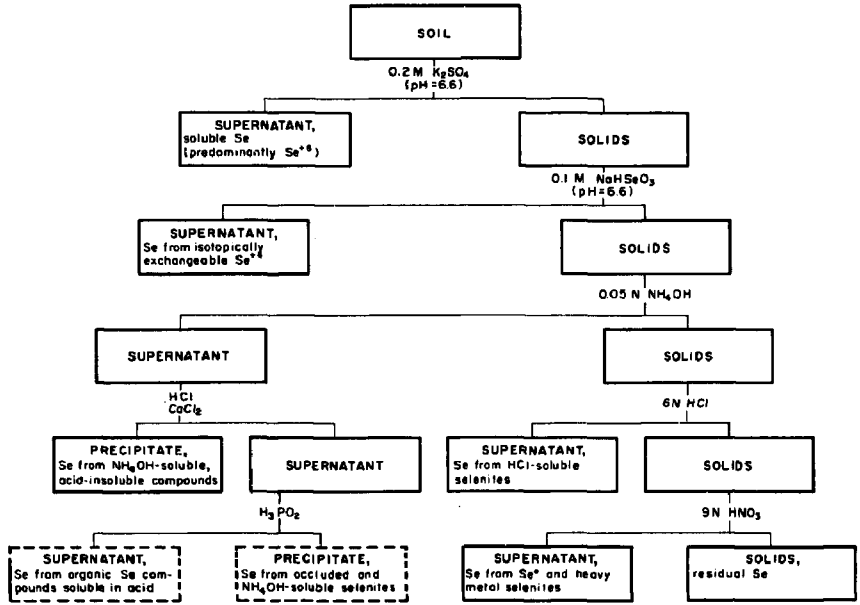

Fig. 2.12. Flow diagram for fractionation of Se-75 from soil (Cary 1967). The selenium precipitated with HCl from the NH_4OH extract should be present solely in organic compounds. The chemical character of selenium in the supernatant from this precipitation is less clear (the two hatched rectangles). The HNO_3-soluble fraction should not contain any organic selenium.

For the sake of convenience it was preferred throughout the procedure to carry out the measurements on aliquots of the supernatants instead of on the precipitates. Aliquots of the Se-75 solution originally added to the soil served as references.

2.4.2. Fractionation of the Water-soluble and the Water-insoluble Selenium Compounds in Soil (Procedure II)

The extraction method described above will usually account for 20 to 60 % of the selenium added to the soil. Hence it was of interest to use another procedure which would account for a larger part of the selenium. For this purpose a fractionation procedure suggested by Cary, Wieczorek and Allaway (Cary 1967) was used. It was based on the existing knowledge of the character of the compounds of native selenium in soil. The steps in the procedure are illustrated in fig. 2.12. The two hatched rectangles were omitted in the present study because the informative value of this part of the fractionation seems questionable.

The procedure was carried out on 1-g samples in centrifuge tubes fitting into the NaI well crystal (see 2.4.4). The samples were counted before the first extraction. For most extractions the soil was shaken overnight with 9 ml solution. After centrifugation the supernatant was discarded and the soil recounted. Each step was repeated until a constant count rate was obtained.

2.4.3. S-35-Labelled Plant Material

The water-soluble sulphur was extracted by a procedure similar to that used for extraction of soluble plant-selenium, and aliquots were used for the determination by means of the activity. After addition of 10 mg sulphate as carrier and precipitation with $BaCl_2$ after acidification with hydrochloric acid the amount of sulphate-sulphur in the extract was obtained as the difference between the total quantity of soluble sulphur and the residual sulphur in the solution. There is a risk of absorption of organic compounds on the $BaSO_4$ precipitate. Washing of the precipitate with hot N HCl will dissolve sulphur amino acids (Kyl 1953). However, no reduction of the count rate for the $BaSO_4$ was found except that corresponding to the dissolved amount of $BaSO_4$. Neither did an inserted precipitation with trichloro-acetic acid influence the result, and it was therefore assumed that no organic sulphur was coprecipitated with the barium sulphate.

2.4.4. Counting Technique

Se-75 in dried plant material was determined on 1-g briquettes (diameter 19 mm) placed in flat-bottomed cylindrical test tubes fitting in the well of a 2-inch NaI crystal. The well was 1″ in diameter and 1 1/2″ deep. The detector was connected with a single-channel analyser with preset count and

preset time modes of operation. The model used was a Philips PW 4280 and supplementary units. Aliquots of the activity mixed with cellulose powder and pressed into 1-g briquettes were used as references. Liquid samples were counted with the same detector. Here references of the same volume were used. With differential counting in the interval 50-500 keV the background was 4 cps and the counting efficiency about 60 %.

S-35 was counted by means of a proportional counter in a set-up with an automatic sample changer (Friesecke-Höffner FH 49). Plant briquettes and cellulose references were of the same weight and thickness so that no correction for self-absorption or counting geometry was needed. The counting efficiency for 1-g briquettes was about 0.1 % and the background 25 cpm.

S-35 in extracts was determined in aliquots evaporated to dryness in an aluminium cup with lens paper fixed to the bottom. In this way a concentration of the solution near the perimeter was avoided. Cups with a diameter of 30 mm could be used in the FH 49 apparatus, where the counting efficiency for these samples was about 2 %. However, both these and larger cups (diameter 45 mm) can be used for counting of low-level samples in a proportional counter with a sample changer developed by Lippert (Lip 1963). The background in this equipment is only 1 to 2 cpm owing to an anti-coincidence circuit, and the counting efficiency with the large cups is about 6 %. This detector was therefore used for all low-level extracts.

2.5. Determination of Inactive Sulphur

The sulphur content in dry plant matter was generally in the range 0.05 to 0.5 %, and the sulphur content in the soil samples was 0.01 to 0.4 %. Various procedures for the combustion as well as for the subsequent sulphur determination were considered. For the combustion a wet ashing, ashing with alcoholic magnesium nitrate and a bomb method were compared. In a few cases the sulphur was precipitated as barium sulphate and determined gravimetrically. However, most samples did not contain enough sulphur for gravimetry. Hence a radiometric method was considered, which we compared with one of the usual turbidimetric methods to decide which was the more suited for routine work. The final choice was the bomb combustion and the radiometric measurement.

2.5.1. Combustion

Wet Combustion. Five to ten g of the dried and ground plant material was combusted in a Kjeldahl flask with 16N HNO_3. When most of the material was dissolved, 5 ml 70 % $HClO_4$ was added and the heating continued until the destruction was finished. The solution was diluted and neutralized, acidified with HCl and filtered. Soil samples were not wet-combusted.

Magnesium Nitrate Ashing. The method is described by Krober and Howell (Kro 1958). One and a half grams of material was mixed in a 100 ml crucible with 15 ml of magnesium nitrate in ethanol (360 g $Mg(NO_3)_2 \cdot 6H_2O$ dissolved in 1000 ml alcohol). The crucible was partly covered with a lid and heated cautiously on an electric plate until the contents were dry. For most plant materials this took a few hours, but with beets more time was necessary as the material easily escaped together with the nitrogeneous vapours. Finally the crucible was left overnight in an oven at 450°C. The ash was dissolved in sufficient hydrochloric acid to make the solution slightly acid. The solution was evaporated, filtered if necessary and the volume made up to 100 ml. In the case of the turbidimetric determination 20 ml of a buffer solution (see 2.5.2) was added before dilution to 100 ml. The sulphur content of the final solution was mostly in the range of 8 to 60 μg per ml.

Table 2.14

Recovery of amino acid sulphur after wet combustion, combustion with alcoholic $Mg(NO_3)_2$ or in bomb with Na_2O_2. Radiometric sulphur determination.

Combustion	Amino acid	μg S added as amino acid	Apparent μg S added as plant material	μg S found	% recovery of amino acid S	% recovery ave.
HNO_3+HClO_4	Methionine	112.8		108.3	96.0	96.4
		173.2		167.5	96.7	
	Cystine	142.5		140.1	98.3	99.4
		126.5		127.0	100.4	
Alc. $Mg(NO_3)_2$	Methionine	108.0		92.0	85.2	
		108.7		90.5	83.4	83.7
		109.2		90.0	82.4	
		111.4	40.2	122.0	73.4	
		109.8	40.2	115.0	68.2	68.4
		108.9	40.2	109.3	63.6	
	Cystine	269.6		252	93.5	
		268.8		253	94.2	93.4
		275.2		254	92.4	
		138.5	40.2	154.5	82.6	
		135.4	40.2	142.5	75.4	81.5
		133.4	40.2	155.5	86.4	
Bomb method	Methionine	107.2		104.0	96.9	
		109.2		105.0	96.0	97.9
		109.2		110.0	100.8	
	Cysteine	135.2		141.0	104.2	
		133.8		131.5	98.4	101.1
		135.0		136.0	100.8	

Bomb Combustion. This was carried out in bombs produced by Janke and Kunkele, Germany, and in accordance with their manual. Depending on the kind of material, 6 to 12 drops of ethylene glycol were placed at the bottom of the bomb before 0.2 to 0.5 g dry material was added. With a few materials it was necessary to add a small piece of filter paper before the sample to initiate the reaction. The material was covered with 8 g Na_2O_2, and the bomb was screwed together and heated on a microburner. After 20 to 40 seconds the fusion took place. The melt was dissolved in sufficient hydrochloric acid to yield an almost neutral solution. The solution was concentrated and filtered if necessary into a 100-ml volumetric flask. Before dilution to the mark, 20 ml of a buffer solution was added for turbidimetric determination. Otherwise the flask was just filled up. The sulphur content of the final solution was in the range 1 to 20 μg per ml.

Control of Combustions. About 50 mg of two sulphur-amino acids was combusted according to the three methods. With alcoholic $Mg(NO_3)_2$ the recovery was not satisfactory (table 2.14). A possible loss due to the small amount of material was proved by mixing the amino acid with the amount of plant material normally used. But the addition did not improve the recovery. Methionine is difficult to oxidize, and it might be that this combustion is not efficient enough, but the loss may also occur in the shape of dust escaping together with the nitrogeneous vapours as indicated by the increase in loss of both acids after addition of plant material.

Soils. The results obtained after the bomb combustion were superior to those obtained after the fusions recommended in "Methods of Soil Analysis" (Bar 1965). Hence the bomb combustion was preferred. The acetate-soluble sulphur (Bar 1965) as well as sulphur extractable with 0.1N HCl (Rasm 1961) were determined. The former is taken to correspond to the readily available amount of sulphate, the latter to the total amount of sulphate.

2.5.2. Methods of Sulphur Determination

Gravimetric Determination. This was used in connection with the wet and the magnesium nitrate ashings. The sulphur was precipitated as $BaSO_4$ and the amount determined by weighing.

Turbidimetric Determination. This was based on a procedure described by Blanchar et al. (Blan 1965). These authors used combustion with HNO_3 and $HClO_4$ and destroyed the perchloric acid with HCl. The solution was diluted, 20 ml of a buffer solution added and the volume adjusted to 100 ml. The buffer consisted of 40 g $MgCl_2 \cdot 6H_2O$, 4.1 g CH_3COONa, 0.83 g KNO_3, and 28 ml 95 % ethanol per litre. Fifty ml of the test solution was stirred with a magnet and 300 mg $BaCl_2$ (20-30 mesh) added. The stirring was continued for exactly one minute, then the liquid was transferred to a colorimeter test tube, and the transmittance at 420 mμ was read exactly two minutes after the addition of $BaCl_2$. The authors found that PO_4-P in amounts of up to 100 ppm did not interfere with the determination of 25 ppm S.

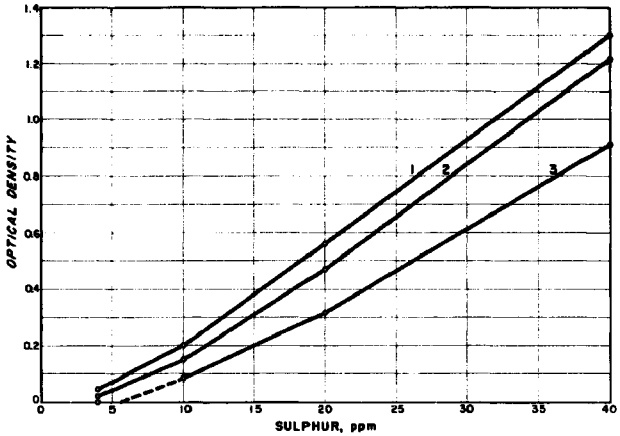

Fig. 2.13. Influence of 2.6 % $MgCl_2$ (curve 2) and 12 % NaCl (curve 3) on the turbidity of $BaSO_4$ suspensions measured with a 1 cm cell. Curve 1 is measured without addition of $MgCl_2$ or NaCl.

After the magnesium nitrate ashing and after the bomb combustion a rather large amount of salt is introduced into the solution. After neutralization the salt concentration will be 2.6 % with respect to $MgCl_2$ or 12 % with respect to NaCl when the final volume is 100 ml. The influence of the salts on the turbidimetric measurement is seen in fig. 2.13. The detection limits in the media water, $MgCl_2$ and NaCl are 3, 4 and 8 ppm respectively, and the sensitivities for concentration above 10 ppm are 0.5, 0.5 and 1.0 ppm respectively.

Radiometric Determination. The precipitation of sulphate with radioactive barium ions and counting of the precipitate has been suggested by Picou and Waterlow (Pic 1963). The authors used Ba-133, a γ-emitter with a half life of seven years. In the present work Ba-131 (γ-emitter, half-life 11 days) was used because it was readily available and because the safety problems are much smaller for the handling of a short-lived isotope. Moreover sulphur determinations were only carried out occasionally, and a series of analyses could be made in a few days with the same references measured each day. The longer half-life of Ba-133 makes this isotope preferable for continuous work because one reference curve for each batch is sufficient for several days, decay corrections being negligible*).

*) The following difficulty encountered with a commercial Ba-133 sample (as a chloride solution) may be useful to others contemplating tracer work with this element. We found that the sample was unusable owing to an appreciable amount of barium activity in an insoluble and dispersed form.

The procedure was the following: Five ml of the test solution from either the bomb combustion or the magnesium nitrate ashing was pipetted into a 12 ml centrifuge tube. One ml of Ba-131-labelled 0.03 M $BaCl_2$ in N HCl was added together with 4 ml ethanol, and the tube was furnished with a rubber stopper and shaken. The amount of activity was about 0.2 μC/ml $BaCl_2$. Alcohol was added to reduce the solubility of the barium sulphate, which in 10 ml water corresponds to 3 μg S and to somewhat more in diluted HCl. The tubes were left for one or two hours at 4°C, then centrifuged for ten minutes at 4500 rpm. The supernatant was discarded, and the surface of the tube and the precipitate were rinsed twice with 1 ml of inactive 0.03 M $BaCl_2$. Picou and Waterlow (Pic 1963) recommended 0.03 M $BaCl_2$ in 60 % alcohol for the rinsing, but no influence on the count rate was found if an aqueous solution was used. The precipitate in the centrifuge tube was counted in a well-type scintillator counter (see 2.4.4). A standard curve was prepared with increasing amounts of sulphate (normally 5 to 200 μg S) in either 12 % NaCl or 2.6 % $MgCl_2$. As seen from fig. 2.14, the range can be

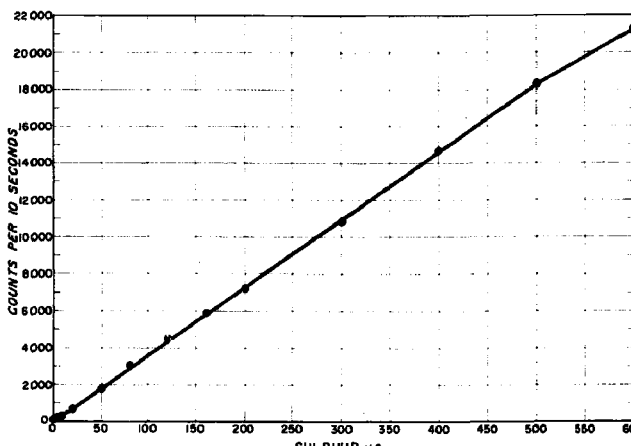

Fig. 2.14. Standard curve for radiometric determination of sulphur as $Ba^{131}SO_4$ measured in a solution containing 12 % NaCl.

extended to at least 600 μg S, and the curve is linear in the range 5 to 400 μg S. The blank value (background deducted) is about 1 cps. The standard deviation of analyses made in duplicate on the same solution is 2.1 % in the range 5-50 μg S and 1.1 % above 50 μg. Contrary to the turbidimetric determination, the radiometric determination is almost independent of the presence of NaCl or $MgCl_2$.

The advantages of the method are the low limit of detection (5 μg S), a sensitivity of 2 μg S at sulphur contents below 100 μg, that the sensitivity is not decreased in salt solutions, and that the method offers a fast procedure. With sixteen tubes in a series one person can make one hundred determinations a day.

It was found that 300 to 1000 μg PO_4-P gives a response corresponding to only 2 to 4 μg S. But the presence of these amounts of phosphate resulted in a considerable increase in the deviation between duplicates. According to Steenbjerg (Stee 1965) the ratio of P to S in cultivated plants generally varies from 0.2 to 10. This usually means that the PO_4-P will not influence the result significantly. Other ash constituents may give rise to a rather loose precipitate of barium sulphate, which may result in a loss through decantation of the supernatant.

The phosphate and other impurities may be removed by scavenging with $Fe(OH)_3$, before dilution to volume of the digested sample. Another counter measure is the use of zirconyl or uranyl acetates. Five ml uranyl acetate (8 g $UO_2(C_2H_3O_2)_2 \cdot 2H_2O$, 10 g CH_3COONa, and 25 ml glacial acetic acid made to 1000 ml with distilled water) was added to 20 ml of the final ash solution. This precipitation was performed in a centrifuge tube so that after centrifugation 5 ml could be drawn off for sulphur determination. For the precipitation of 1000 μg P about 2.5 ml of the reagent was necessary. Together with the phosphate both reagents removed the constituents causing a loose $BaSO_4$ precipitate. As precipitation was necessary only in a minor part of the samples, it was most convenient to use uranyl acetate because this can be done as a single addition after dilution to volume.

2.5.3. Comparison of the Methods

Results obtained on plant materials with two or more of the procedures described are shown together with their detection limits in table 2.15. In most cases the alcoholic $Mg(NO_3)_2$ combustion shows a lower sulphur content than the bomb method. The difference is in accordance with the recovery of 70 to 90 % for pure amino acids (table 2.14), which was explained partly by incomplete oxidation and partly by mechanical loss during the combustion. The result of the sulphur determination seems independent of the method used, but that was to be expected because all three determinations are based on the same reaction.

The concentration required for detection of the sulphur by the three methods varies forty times owing to sample size and measurement. It is seen (table 2.15) that the radiometric determination is six times as sensitive as the turbidimetric one. The radiometric method was therefore preferred, the more so as it is very suitable for a large number of samples. The bomb method was chosen for combustion in preference to wet combustion because the former method is fast and clean, and because wet combustion cannot be used for all

Table 2.15

Comparison of methods for sulphur determination

		Method			
		Bomb combustion, turbidimetry	Bomb combustion, radiometry	Alc. $Mg(NO_3)_2$, radiometry	Alc. $Mg(NO_3)_2$, gravimetry
Limitations of the methods	Sample weight	0.3 g	0.3 g	1.5 g	1.5 g
	Detection limit	8 µg/ml	1 µg/ml	1 µg/ml	0.5 mg
	Corresponding conc. in dry matter (100 ml ash soln.)	2700 ppm	350 ppm	70 ppm	350 ppm
Examples, ppm S	Turnips	6380	6500	5470	5450
	Onion, bulb		3280	2600	
	Rye grass	2640	2580	2730	2880
	Barley, grain (0.5 g)	1300	1340	1035	900
	Sugar beet		<400	460	

kinds of materials. In a few samples with very low sulphur concentrations alcoholic magnesium nitrate combustion was used despite the apparent recovery of only about 80 %.

2.6. Pot Experiments

An outline of all pot experiments is given in table 2.16, which also includes experiments from which results have been published (Bis 1969, Gis 1970b). Only the fertilization and the tracer application will be summarized below. Data for the soils including results of the selenium and sulphur determinations are given in table 3.2.

A standardized gardening soil (soil No. 7) was used for pot experiment No. 1. Six edible and two wild Allium species were grown in 5 kg pots. Four of the species were grown from bulbs, the others from seeds.

For experiments Nos. 2 to 5 the soil received a basic dressing of 0.75 g NPK-fertilizer (16-5-12), 25 mg $CuSO_4 \cdot 5H_2O$, and 20 mg $MnSO_4 \cdot H_2O$ per kg. For experiments Nos. 7 to 9 a basic dressing was used per kg soil of 0.30 g $Ca(H_2PO_4)_2 \cdot H_2O$, 0.24 g $Mg(NO_3)_2 \cdot 6H_2O$, 0.36 g NH_4NO_3, 0.54 g K_2SO_4, 20 mg $MnSO_4 \cdot H_2O$, and 4 mg borax. In experiment 6 the sulphate and nitrogen levels were varied and the potassium level maintained constant through the addition of varying amounts of K_2SO_4, KNO_3 and NH_4NO_3. For experiments 2, 4, 5, and 6 the fertilizers were weighed out for each portion of soil. In the other experiments the whole amount of a soil was mixed with the basic fertilizers.

Outline of the pot experiments

Expt. No.	Main purpose	Month of sowing, year	Kg soil per pot	No. of replicates	Tracers	Soil No.	Oxidn. state of Se	ppm Se added	ppm SO_4-S added	ppm N added	Test plants	Results in chapter No.
1	Se in Allium species	5-65	5	2	None	7		0			8 Allium species	3
2	Influence of soil	5-66	20	3	Se-75	1 to 6	+4, +6	0.5	7	31	Clover barley, mustard	4
3	Influence of Se-conc. and plant species	4-66	2	3	Se-75	2	+4,+6	0.1-0.5-2.5	7	31	Ten species	4
4	Influence of Se-compound	4-66	20	3	Se-75	2	0,+4,+6	See table 4.7	7	31	Clover, barley, mustard	4
5	Influence of Se-compound and soil	10-67	20 2.5	3 6	Se-75 Se-75	2,5 2,5	+4,+6 +4,+6	0.5 0.5	7 7	31 31	Mustard, Radish	4 4
6	Influence of S, N and org. matter	12—65	1	6	Se-75	2,5	+4,+6	0.7	5-255	25-300	Radish	4
7	Influence of Se on S-uptake	6-66	1	6	S-35	2	+4,+6	0-2	100	150	Radish	6
8	As for expt. 7	11-66	1	6	S-35	2	+6	0-10	100	150	Radish, rye grass	6
9	As for expt. 7	12-67	1	6	S-35	2	+4	0-50	100	150	Radish	6

Selenium-75-labelled compounds were prepared for experiments 2 to 6. Se-75 as H_2SeO_3 with a high specific activity was used for the preparation. For the labelling of selenates the active selenite in a neutral to slightly alkaline solution was oxidized by boiling with bromine.

The readily soluble selenium salts were labelled by adding the activity in the proper oxidation state to a solution of the inactive salt. The other selenites and elemental selenium were precipitated from labelled K_2SeO_3 and the respective chlorides. Cellulose powder was added to provide a precipitate more easily mixed with the soil. $CuSeO_4$ and $BaSeO_4$ were precipitated from the chlorides and labelled K_2SeO_4 by means of ethyl alcohol. It was controlled that the specific activities of the compounds for an experiment were identical, and that the oxidation state was correct.

The specific activity used in seven of the experiments was from 2 to 10 μC/mg Se. In experiment 3 the specific activity was varied to obtain a suitable count rate. The highest specific activity was 100 μC/mg Se used together with the lowest concentration of selenite.

Aliquots of the labelled selenium salts in solution were evaporated on 20 to 50 g sand. The sand samples as well as the cellulose-diluted compounds were mixed with a small part of the soil before being added to the whole portion. A portion was either meant for a single pot (20 kg soil) or for all the replicates of a treatment (6 to 15 kg soil).

Labelled sulphate was applied in experiments 7 to 9. Carrier-free S-35-labelled H_2SO_4 was added to a solution of $CuSO_4$, aliquots were added to sand and mixed with the soil as above. Ten μC S-35 with a specific activity of 2 μC/mg S (as $CuSO_4$) was used per kg of soil.

In experiments Nos. 2 and 4 the pots were placed in a screened outdoor enclosure. An exception was the clover in experiment No. 2 because it was grown for one and a half years and therefore placed in the greenhouse during the winter. In the other experiments the pots were placed in a greenhouse.

2.7. Field Experiments

Field experiment No. I (1965-66). The purpose of this experiment was to follow the influence of the amount of precipitation and of the season on the selenium concentration in plants. The experiment was laid out at Risö in the spring of 1965 in a field with white clover and rye grass established in 1964. No selenium was added to the soil. Fertilizers had last been added in the autumn 1964. Three out of nine plots in a Roman square were covered with glass 70 cm above the ground. These plots were supplied with as little water as possible for the growth. Three other plots received the precipitation, and three plots got the precipitation supplied with watering. The water content of the soil was controlled by measurement of the neutron absorption with a Nuclear Chicago Corp. moisture tube (model P-19) (Haa 1961). About 1 m^2

in the centre of each plot was harvested four times during the summer of 1965 and four times during the following summer. In the winter the covering was removed.

Field experiment No. II (1965): For comparison of the selenium concentration in different crops grown on a non-selenized soil, a series of 17 agricultural species were grown in the field at Risö. Three plots of 2 m^2 each were used for each crop in a randomized lay-out. 400 kg/ha of NPK (16-5-12) had been added.

Field experiment No. III (1966-67): This experiment has been described elsewhere (Gis 1970b). Suffice it here to say that the experiment was laid out in plots of 2 m^2 (in duplicate) on a soil similar to soil No. 5 (table 3.2) and run for two years. Plots with beets, potatoes and brassica species received each year 700 kg NPK/ha, the other plots 400 kg/ha. Selenium compounds (not labelled) were applied the first year only. The applications were: none, 12.5 kg Se°/ha, 1.25 kg Se as K_2SeO_3, 0.25 kg as K_2SeO_4, and 0.25 kg/ha as $BaSeO_4$ (1 kg/ha~0.4 ppm roughly). Each year soil samples were taken after harvest at different depths in order to measure the leaching.

3. SELENIUM IN DANISH CROPS AND SOILS

In Denmark there is no evidence of serious selenium deficiency with frequent and heavy losses of livestock. Nevertheless, many cases have been recorded where lack of selenium might be the cause of losses (Rasb 1965). In newborn pigs the main symptom is high mortality, and an autopsy shows that the muscles are light coloured. Pigs affected with this white muscle disease are found especially at farms along the Jutland glacial ridge (Rasb 1965). Another disease, called stiff-lamb disease, has been observed on the west coast of Jutland (Løn 1965). When selenium was supplied alone or together with vitamin E, the effect was beneficial (Lud 1965, Løn 1965, Rasb 1965).

From other countries it is known that selenium deficiency symptoms will appear when the Se concentration in the food on a dry basis is below 0.03 to 0.05 ppm and perhaps already below 0.1 ppm. According to this the minimum requirement was set (see 1.3.2) at 0.05 to 0.1 ppm and the desirable level at 0.1 to 0.3 ppm.

When the present work was undertaken, the selenium concentration in Danish crops was unknown, and therefore a decision was taken to survey the contents in representative agricultural crops as well as in a few wild plant species. The most important crops for feeding are barley, pasture species and root crops. Barley grain and pasture species were studied most comprehensively. The samples were collected from various parts of the country, barley over a period of three years, pasture species twice during one summer. In addition to these samples a series of root crops and some wild plants were included in the survey. Further experiments were carried out to augment the data with information about the variation of the selenium content with the precipitation and through the season and among different crops.

3.1. Sampled Material

Most of the crop samples were taken at the State Experimental Stations in connection with experiments where old and new plant varieties were compared. The growth conditions were comparable with respect to fertilization and other treatments. As a control some samples were also taken from private farms. The sampling was done by trained people in connection with the normal harvest. The geological origin of the soils is shown in fig. 3.1, in which also the sampling locations are indicated.

Barley grain (1964, 1965, 1966). In 1964 samples of the varieties Carlsberg II, Weibulls Ingrid, Weibulls Rika, Svalöf Bonus, and Svalöf Pallas were collected at the stations St. Jyndevad, Studsgaard and Ødum. Samples of Bonus and Pallas were also obtained from three private farms in each area. In addition to these samples barley grain was analysed from ten farms compris-

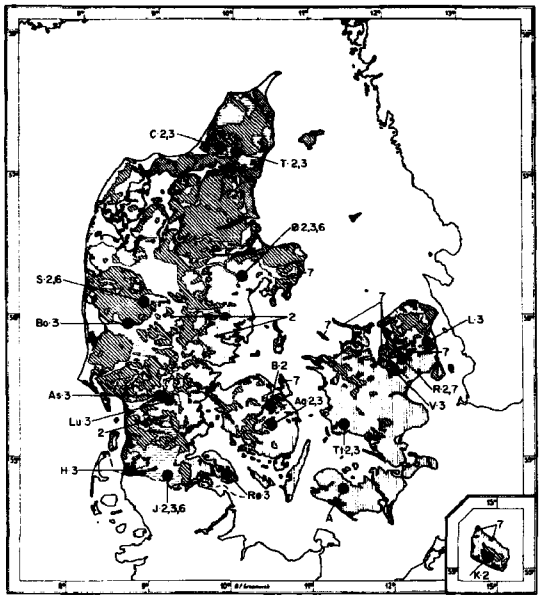

Fig. 3.1. The locations of sampling of the plant material. The letters show the location of the Experimental Stations:

A	= Abed	H	= Højer	Rø	= Rønhave
As	= Askov	J	= St. Jyndevad	S	= Studegaard
Aa	= Aarslev	K	= Kannikegaard	T	= Tylstrup
B	= Blangstedgaard	L	= Lyngby	Tt	= Tystofte
Bo	= Borris	Lu	= Lundgaard	V	= Virumgaard
C	= Centralgaarden	R	= Risø	Ø	= Ødum

The numbers refer to the sampled crops and are the same as used in the tables and the figure containing the analytical results:

2: barley grain (fig. 3.2)
3: pasture species (table 3.3)
6: root crops (table 3.6)
7: wild plants (tables 3.7 and 3.8)

ed by the "Husbandry Health Registration" (Rasb 1968), mainly farms where indications of selenium deficiency had been noted.

In 1965 the same five varieties were sampled from the same three stations and from Blangstedgaard, Tystofte and Risø. Again the material was supplemented with samples from private farms.

Finally in 1966 barley grain from Blangstedgaard, Aarslev, Tylstrup, Centralgaarden, Aakirkeby, and St. Jyndevad was analysed. The varieties that year were Weibulls Ingrid, Weibulls Rika, Svalöf Pallas, Proctor, Impala, and Vada. In addition grain samples were taken at two private farms on Funen. During these three years a total of 31 farms were included with from one to eleven samples each.

Pasture species (1965). Samples were taken in June - July and again in August - September. Thirteen Experimental Stations were included. The species were rye grass (late Øtofte Dux III), timothy (Øtofte A III), white clover (Pajbjerg Milka), and lucerne (Du Puits). Besides sampling was done in four untypical grazing areas with mixtures of grasses. One of the soils was rich in sulphur The areas were marine foreland at Højer, reclaimed sea floor in the Lammefjord and former meadows at Aanum and Stauning. The plant samples were mostly dried at the Experimental Stations.

Root crops (1965). Sampling was done at three Experimental Stations and comprised five varieties of sugar beets, two of swedes and two of potatoes.

Wild plants (1965). Sampling was mostly done at the blooming stage and only of herbages. No attempt was made to make the sampling representative of wild plants in general, but the following points were considered: (1) The selenium concentration in the two Danish Astragalus species as compared with other Papilionaceae, (2) the concentration in members of the Allium group because they are rich in sulphur, and (3) the possibility of a difference in selenium concentration due to the type of land. Most of the sampling was done in Zealand *).

Soil samples. Two series of soil samples were analysed for selenium and sulphur. One series consisted of six soils used for pot experiments with selenized soils. The other series comprised eleven soil samples taken in permanent grass areas.

3.2. Results

3.2.1. Soils

The selenium content in the investigated soils varied from 0.1 to 1.6 ppm (tables 3.1 and 3.2). As is the case with other elements, the selenium content seems higher in soil samples taken from grass areas (table 3.1) than in samples from areas in rotation. The total sulphur content varies from 100 to 4000

*) The author is very grateful to Professor M. Køie, The University of Copenhagen, for procuring most of the Astragalus and Allium species.

Table 3.1

Selenium and sulphur content of some Danish soils. The samples are from permanent grass areas not in rotation. The data are on an air-dried basis.

Experimental Station	pH (in H_2O)	ppm selenium		ppm sulphur		
		Total	H_2O-soluble	Total	Readily available SO_4-S	Total SO_4-S
Abed	7.9	1.40	0.030	2600	22	88
Tystofte	7.9	0.45	0.026	320	27	40
Ledreborg	7.7	0.29	0.008	500	5	10
Virumgaard	6.1	0.21	0.010	350	9	30
Kannikegaard	5.9	0.32	0.009	360	25	55
Blangstedgaard	6.1	0.85	0.024	940	25	55
Ødum	6.2	0.43	0.027	230	11	38
Askov	6.7	1.44	0.007	700	27	40
St. Jyndevad	5.4	0.20	0.013	220	8	43
Studsgaard	4.2	0.28	0.008	290	10	25
Tylstrup	6.0	0.45	0.013	1200	40	100

ppm, and it is mostly a little higher in the samples from grass areas. The figures for sulphur agree with those of Dam Kofoed and Lindhard (Kof 1968). The sulphur - selenium ratio varies in both sets of data between 500 and 3000, on the average it is 1400. This is only half or less of that known from the sediments (tables 1.1 and 1.2) and indicates that Se^{+6} has not been the dominating oxidation state during the soil formation. The amount of selenium extractable with water constituted from 0.5 to 6.5 % of the total, on the average 3.4 %. Though the figures for the soluble selenium are very approximate, it is seen that a diluted solution of $CaCl_2$ may bring up to twice as much selenium into solution (table 3.2). The total amount of sulphate constitutes from 2 to 20 % of the total sulphur and the readily available sulphate about half as much. This agrees well with the early data of Christensen from 1917 (cited in Stee 1965). The linear correlation coefficient between total sulphur and total selenium in tables 3.1 and 3.2 is 0.76 (soil No. 6 in table 3.2 is not included because this soil is very special). The correlation coefficients between other sets of the sulphur and selenium data are only 0.57 or less.

The concentration of selenium in the soils corresponds well with the range reported from abroad. Swaine (Swa 1955) stated 0.1 to 2 ppm both for toxic and non-toxic soils. For eleven non-toxic soils in the U.S.A. the range was 0.01 to 2.5 ppm (Sla 1937) (but these data may well be below the true values), and most soils in New Zealand contain 0.1 to 2 ppm (Wat 1962). Soils containing less than 0.45 ppm were generally consistent with a response by livestock to selenium supply (Wat 1962). The present work provides the

Table 3.2

Data for soils used in pot experiments. The six samples are from fields in rotation.

Soil No.	Locality	Soil type	Particles <0.002 mm %	CEC meq. pr. 100 g	Org. matter %	pH (H_2O)	ppm selenium			ppm sulphur		
							Total	H_2O-sol.	Sol. in 0.05 N $CaCl_2$	Total	Readily available SO_4-S	Total SO_4-S
1	Tarm	Poor sandy loam	4	18.0	2.9	4.8	0.11	0.007	0.02	105	5	15
2	Li. Valby	Loamy sand	9	15.2	2.8	5.7	0.12	0.002	0.003	240	10	25
3	Borris	Loamy sand	9	15.1	3.2	5.9	0.19	0.005	0.007	200	7	30
4	Ødum	Sandy loam	17	14.2	2.1	7.3	0.19	0.008	0.011	140	15	30
5	Riso	Sandy clay loam	21	17.3	2.1	7.4	0.13	0.003	0.004	225	10	25
6	Lammefjord	Muck soil	31	47.4	12.8	7.6	1.6	0.010	0.02	4500	270	510
7		Standard-soil for gardening			15.0	6.8	0.4					

same distribution pattern. Samples from ten farms recorded by the "Husbandry Health Registration" (Rasb 1968) all contained less than 0.04 ppm Se.

Differences with respect to selenium content between barley varieties from the same farm were small, mostly less than 30 %, and no systematic difference between the varieties was found. The difference in selenium concentration between two years was no more than a factor of 2 in grain from the same station. The extent to which the weather may have played a role in this connection cannot be seen.

It was remarkable that only grain from two areas contained more than 0.08 ppm Se, and as mentioned above these areas can hardly be considered normal from an agricultural point of view. In general it seems that most of the Danish barley grain will contain 0.02 to 0.05 ppm Se and a minor part a little more or less.

3.2.3 Pasture Species

The four pasture species contained 0.1 to 0.2 ppm Se (table 3.3). Blangstedgaard and Ny Vraa were not among the localities because no pasture experiments were run there. Of the four species, white clover may contain somewhat more selenium than the other pasture species. This would agree with the behaviour of other elements. It is common that clover and lucerne contain more of for instance, K, Na, Ca, Mg, and Fe than do rye grass and timothy (Kristensen 1952, cited in Stee 1965). The four grass samples taken

Table 3.3

Selenium content in pasture species from different parts of Denmark. The samples were taken in 1965 in June — July and August — September, mostly as the second and fourth cut. ppm Se in the dry matter.

Species	Field established in	No. of		ppm Se	
		Localities	Samples	Range	Ave.
Rye grass (Lolium perenne)	1963	8	11	0.082-0.152	0.12
Timothy (Phleum pratense)	1963	7	10	0.100-0.168	0.14
White clover (Trifolium repens)	1963	7	11	0.108-0.207	0.17
Lucerne (Medicago sativa)	1964	6	7	0.084-0.195	0.13
Grasses (no botanical analysis)		4	4	0.173-0.251	0.21

Table 3.4

Selenium concentration in white clover — grass grown in the field at Risø with three levels of water supply. The field was sown in 1964. The following two years the plants were cut four times each summer. (Field experiment No. I).

		1965				1966				Average of ppm Se		Total yield g/m²	
		May 11	June 14	July 19	Sept. 15	May 17	June 15	July 26	Oct. 6	1965	1966	1965	1966
Large water supply	Se, ppm	0.216	0.183	0.158	0.073	0.043	0.044	0.060	0.059	0.156	0.052		
	Yield of dry matter, g/m²	80	800	190	220	40	120	190	110			1290	460
Precipitation*)	Se, ppm	0.204	0.156	0.152	0.090	0.060	0.063	0.027	0.065	0.151	0.055		
	Yield of dry matter, g/m²	70	360	180	170	50	120	100	60			780	330
Small water supply	Se, ppm	0.194	0.166	0.142	0.090	0.055	0.069	0.042	0.068	0.148	0.061		
	Yield of dry matter, g/m²	70	110	80	15	30	80	40	10			275	110
Average for the three water levels	Se, ppm	0.205	0.168	0.149	0.064	0.053	0.059	0.043	0.064				

*) 346 mm in the period April 1st to Oct. 1st, 1965
352 mm in the period April 1st to Oct. 1st, 1966

Table 3.5

Water content of the Risø soil during the summers of 1965 and 1966. Large water supply was obtained from precipitation plus watering, small water supply was obtained in glass-shielded plots watered when necessary. The covering was removed in the winter. (Field experiment No. I).

	Depth. cm	% water in the period between two cuts							
		1965				1966			
		-1'	1'-2'	2'-3'	3'-4'	-1'	1'-2'	2'-3'	3'-4'
Large water supply	25	27.3	22.8	21.5	25.6	29.4	24.3	26.6	29.1
	50	30.3	29.1	27.0	28.2	31.2	29.9	30.2	31.6
Precipitation	25	26.6	18.5	14.2	20.2	28.7	21.4	13.4	20.8
	50	26.1	22.0	15.8	17.4	27.6	25.0	20.1	19.5
Small water supply	25	24.0	14.4	10.2	11.7	29.4	20.2	13.7	12.9
	50	27.8	23.8	19.9	20.0	30.2	28.0	22.9	21.2

from other types of pasture areas contained most selenium. No significant difference in the selenium content was seen between samples from moraine clay and from more sandy soils, nor did the concentration in June - July differ from that in August - September. In a field experiment (No. I) at Risø, however, a considerable decrease was found in white clover from May to September in the first year of use, while the following summer the selenium content was fairly constant, but lower, despite a much lower yield (table 3.4). The concentration varied through the season also in other investigations of white clover and grasses in already existing swards. In some cases a considerable decrease in selenium content with the cut number was found (Dav 1966a and 1966b), while in other cases the content was fairly constant in the successive cuts (Gra 1965, Les 1968). Whether the different results are related to changes in the dry matter percentage or in the yield cannot be seen.

In Australia a negative correlation between the rainfall and the selenium concentration in pastures has been found (Gar 1963). A correspondingly higher incidence of NMD after rainy summers has also been reported (Gar 1962, Oks 1965). In the above-mentioned experiment with the grass - white clover sward (field experiment No. I) three levels of water supply were maintained during two summers. The second year there was somewhat more water in the soil. The water content of the soils varied between 10 and 30 %, depending on the treatment and the time of the year (table 3.5). The curves showing the utilization of the selenium (total uptake vs. yield) did not differ significantly for the three treatments. The concentration seemed independent

of the water supply, though the yield differed by a factor of five (table 3.4). Similarly, in a pot experiment Ehlig et al. found for lucerne, but not for timothy (Ehl 1968) that the concentration did not depend upon the yield.

As a whole the pasture species from fourteen localities contained 0.1 to 0.2 ppm Se, and the samples from the Risø field in 1966 were the only samples with concentrations below 0.08 ppm.

3.2.4. Root Crops

The selenium concentration in root crops varied very much with the species (table 3.6). Swedes contained most selenium as could be expected in a

Table 3.6

Selenium content of root crops harvested 1965 at Studsgaard, St. Jyndevad and Ødum

Crop	No. of samples	% dry matter	ppm Se in dry matter	
			Range	Ave.
Swedes (Brassica napus)	4	12.5	0.22- 0.62	0.35
Sugar beets (Beta vulgaris)	9	20.4	0.025-0.107	0.051
Potatoes (Solanum tuberosum)	5	21.6	0.014-0.021	0.017

member of the sulphur-rich Cruciferae family. Swedes also show a higher concentration of the micronutrients than the other two root crops. Potato tubers had the lowest Se concentration. As seen from the width of the ranges, the concentration in a species varied somewhat more between the localities than was the case with the pasture species.

3.2.5. Wild Plants

A number of wild herbacious plants had a selenium content that was comparable with that of the cultivated plants (table 3.7). When the plants are grouped after the place where they grow, it is seen that the coastal plants contain less selenium than plants from agricultural and forestal areas (table 3.8). The two Danish species of Astragalus, A. glycyphyllus L. and A. danicus Retz., were represented by five samples with an average selenium content of 0.08 ppm. Thus these species do not differ appreciably from other plants with respect to selenium absorbance properties as is the case with some of the American Astragalus species.

Table 3.7

Selenium content in wild plants belonging to different botanical families. Sampled at flowering (1965). ppm Se in the dry matter.

Botanical family	No. of species	No. of localities	No. of samples	Part of plant	ppm Se	
					Range	Ave.
Compositae	6	5	11	Top	0.04-0.24	0.10
Papilionaceae	5	6	8	—	0.04-0.11	0.08
Liliaceae	4	6	9	—	0.04-0.24	0.09
—	4	6	9	Bulb	0.03-0.11	0.07
Euphorbiaceae	2	2	3	Top	0.04-0.05	0.04
Miscellaneous	6	4	6	—	0.07-0.19	0.12
Total	23	15	37	Top	0.04-0.24	0.09

Table 3.8

Selenium content in wild plants growing on three types of land. ppm Se in the dry matter of the top of the plant.

Type of land	No. of localities	No. of species	No. of samples	ppm Se	
				Range	Ave.
Forest	5	5	10	0.05-0.24	0.12
Headland, roadside, etc.	4	7	17	0.04-0.24	0.10
Sea shore	6	12	10	0.04-0.09	0.06

3.2.6. Allium Species

The selenium content of some cultivated Allium species and of two wild species was determined in a pot experiment (table 3.9). Of the cultivated species the leek had the greatest concentration. In two cases a greater concentration was found in the bulb than in the top. The new bulbs of the two wild species contained five to ten times as much selenium as the old bulbs. In this well-fertilized and rather organic soil, A. scorodoprasum reached a selenium content of 1.2 ppm, which is much more than the edible bulbs contained and altogether the highest concentration found in plants grown on a non-selenized Danish soil. A. scorodoprasum normally grows close to the sea.

Table 3.9

Selenium in the dry matter of some Allium species grown in a soil standardized for gardening (Soil No. 7). (Pot experiment No. 1).

Species	Common name	ppm Se in original bulb	ppm Se	
			Top	Bulb
A. cepa (4 varieties)	Onion		0.12-0.15	0.08-0.30
A. ascalonicum	Shallot	0.04	0.12	0.11
A. fistulosum	Welsh onion		0.16	0.08
A. sativum	Garlic	0.14	0.21	0.12
A. schoenoprasum	Chives		0.26	0.12
A. porrum	Leek		0.40	0.38
A. vineale	Crow garlic	0.04	0.19	0.18
A. scorodoprasum	Rocambole	0.11	0.7	1.2

3.2.7. Comparison of Agricultural Plants with Respect to Their Selenium Content

Seventeen plant species grown in the field at Risø showed variation in selenium concentration from 0.02 ppm in potato tubers to 0.2 ppm in the top of swedes (table 3.10). The following two years a similar series of crops was grown in another Risø field (table 3.11). In this experiment a selenium range from 0.03 to 0.7 ppm was found. The results varied somewhat between the two years most for potato tubers where the content was 0.2 and 0.03

Table 3.10

Selenium and sulphur content in different crops grown in the field at Risø. (Field experiment No. II). ppm Se in dry matter.

Botanical family	Crop	Part of the plant	ppm Se	ppm S
Cruciferae	Turnip	Top	0.17	6600
		Root	0.12	4600
	Swedes	Top	0.19	5500
		Root	0.08	3250
	Rape	Seed	>0.06 *)	10400
		Pod	0.12	4400
		Straw	0.10	3650
	White mustard	Seed	>0.07 *)	13500
		Pod	0.11	4300
		Straw	0.10	3850
Compositae	Chicory	Top	0.13	4550
		Root	0.06	950

Table 3.10, continued

Botanical family	Crop	Part of the plant	ppm Se	ppm S
	Lupin (July)	Green	0.09	3300
	Lupin (Sept.)	Seed	>0.11 *)	5000
		Pod	0.06	1050
		Straw	0.07	5650
Papilionaceae	Common vetch	Seed	>0.10	2200
		Pod	0.12	800
		Straw	0.13	3150
	Bird's-foot trefoil	Green	0.11	2400
	Red clover, 1st cut	—	0.07	1400
	Red clover, 2nd cut	—	0.09	2450
	Black medick	—	0.08	1550
	Rye grass, 1st cut	Green	0.15	2950
	2nd cut	—	0.10	2800
	Oats	Grain	0.04	1500
		Straw	0.05	1400
Graminaceae	Barley	Grain	0.04	1200
		Straw	0.08	1550
	Rye	Grain	0.03	1100
		Straw	0.05	1550
	Wheat	Grain	0.03	1150
		Straw	0.06	1000
Chenopodiaceae	Sugar beet (2 varieties)	Top	0.06	3400
		Root	0.06	700
Solanaceae	Potato	Top	0.11	2450
		Tuber	0.02	1100
Correlation coefficient between Se and S: $r = 0.69$				

*) Wet combustion with HNO_3 alone. Hence the results are only about half the true value.

Table 3.11

Selenium content in the dry matter of some crops grown in the field at Riss in 1966 and 1967. Field experiment No. III (Gis 1970b).

Botanical family	Crop		ppm Se	
			1966	1967
Cruciferae	Turnip	Top	0.5	0.38
		Root	0.73	0.16
	Marrow-stem kale		0.38	
	Swedes	Top	0.20	
		Root	0.38	
	White mustard	Seed	>0.14	>0.17
		Straw	0.13	0.12
Papilionaceae	Black medick	1st cut	0.16	0.15
		2nd cut	0.36	0.09
	Red clover	1st cut	0.10	0.10
		2nd cut	0.23	0.10
	Lucerne	1st cut	0.09	0.16
		2nd cut	0.12	0.16
Graminaceae	Rye grass	1st cut	0.14	0.09
		2nd cut	0.35	
	Oats	Grain	0.05	
		Straw	0.08	
	Barley	Grain	0.03	0.04
		Straw	0.15	0.04
	Rye	Grain	0.07	
		Straw	0.08	
	Wheat	Grain	0.1	0.06
		Straw	0.13	0.04
Chenopodiaceae	Sugar beet	Top	0.12	0.14
		Root	0.06	0.03
Solanaceae	Potato	Top	0.29	0.09
		Tuber	0.21	0.03

ppm respectively. As is known of many of the plant nutrients the green part of the plants generally contained more selenium than the underground part. The grain contained less than the straw as is the case for the nutrients (except N and P) when the supply of fertilizers is low to moderate (Stee 1965).

The concentration differs somewhat with the plant family. Other comparisons of plant species with respect to selenium concentration comprising several species have been made on seleniferous or selenized soils (see 1.2.1). As far as these and the present results are comparable the general trend is that members of the Cruciferae family are the richest in selenium,

Table 3.12

Average sulphur and selenium concentrations of various crops grown in Danish soils.

Crop	No. of samples	ppm in dry matter	
		S	Se
Sugar beet	9	430	0.051
Barley, grain	21	966	0.068
Potato, tubers	5	1220	0.017
Timothy	5	1880	0.127
Rye grass	11	2610	0.119
Lucerne	5	2765	0.149
Clover - rye grass, 1st cut	6	3080	0.200
3rd cut	6	2180	0.154
4th cut	6	2790	0.083
Swedes, root	4	2590	0.35
Onion, top	7	4610	0.21
bulb	9	5720	0.17
Correlation coefficient between Se and S : r = 0.45			

and that beets, potatoes and grain the poorest just as is generally the case with the nutrients. The variation in the concentration in agricultural and gardening crops is usually not more than a factor of 10.

Comparison of the selenium and sulphur concentrations of the plants in table 3.10 resulted in a correlation coefficient of 0.69. The seed samples were not included. A further comparison of the concentrations of the two elements was made with part of the collected samples and the Allium species (table 3.12). It is seen that the sulphur concentration in successive cuts of white clover - rye grass did not decrease with the cut number as did the selenium concentration (table 3.4). The correlation between the selenium and sulphur content in the material in table 3.12 was poor (r = 0.45). The calculation was made on the averages for each crop. The ratio between the sulphur and the selenium concentrations varies (tables 3.10 and 3.12) between 7,000 and 80,000, but the range is much greater in table 3.12 as reflected in the correlation coefficient. This difference between material from all over the country and material from a single field points to the possibility that the ratio might be fairly constant in so far as the amounts of available sulphur and selenium are constant. This assumption is further supported by the results of Fleming (Fle 1962a) who (in a pot experiment with a seleniferous soil) found a variation in the S:Se ratio comparable with that in our field experiment (table 3.10).

Table 3.13

Selenium concentration in some typical Danish crops. Summary of the results.

Crop	No. of samples	ppm Se in dry matter	
		Range	Ave.
Potato, tubers	7	0.014-0.03	0.019
Barley, grain	39	0.015-0.08	0.037
Sugar beet	13	0.025-0.11	0.051
Pasture species	66	0.03 -0.25	0.14
Swedes, root	6	0.08 -0.62	0.31

3.3. Discussion

The results for some agricultural crops are summarized in table 3.13. The samples are primarily those from the State Experimental Stations and the few exceptional results for barley grain are not included. Though the number of samples and localities are restricted, the results for barley grain and pasture species may be considered representative, because the State Experimental Stations represent the most typical agricultural soils in our country, and because samples from private farms were not different from those from the State Farms. The figures for the other three species in the table are only indicative. In this material no difference in concentration was seen that was related to the soil type.

The selenium concentration in crops is not so low in Denmark as for instance in parts of the U.S.A., in New Zealand and in Sweden. In the U.S.A. 80 % of the pastures from about half the country contain less than 0.1 ppm Se in the dry matter, and NMD in sheep and cattle is widespread (Kub 1967). In Sweden the selenium concentration in grain seems to be substantially lower than in Denmark. In grain samples from ten localities only samples from one locality contained more than 0.02 ppm Se (Lin 1968), and NMD in pigs is common. Also in a few pasture samples the concentration was lower than in Denmark (Lin 1970).

When the present results are compared with the requirements of livestock it should be borne in mind that the necessary amount of selenium is to some extent increased with a fall in the vitamin E supply and with the amount of unsaturated fatty acids. Further there is a possibility of loss of selenium during storage under bad conditions or during hay or silage production. Finally a lower concentration is possible in years with very rainy and cold summers. Besides, the selenium level may decrease with intensified cropping or grazing as has happened in New Zealand (Har 1961). A change in the selenium concentration may also occur as a consequence of the use of

NPK-fertilizers instead of superphosphate because this means a decrease in both the sulphur and the selenium supply.

The minimum selenium requirement of ruminants is 0.05 to 0.1 ppm and the desirable supply 0.1 to 0.3 ppm in the dry fod. Though pigs may be less susceptible to selenium deficiency than ruminants (Wri 1966), a lower minimum requirement has not been stated for this animal.

Barley grain constitutes an important part of the food for the pigs. Supplementarily, for instance skimmed milk, potatoes and meat-bone-meal are used. From table 3.13 it appears that the selenium supply from the grain alone will generally be insufficient. In Danish whole milk from 0.015 to 0.12 ppm Se has been found on a fresh-weight basis or 0.12 to 0.78 in the dry matter (Bis 1970). The content in skimmed milk is somewhat higher. In other countries from 0.02 to 0.4 ppm has been found, depending on the type of milk powder (Fin 1967, Kie 1968, Lin 1968). An important part of the food for cattle is root crops and pasture species. At least part of the year supplements are given such as skimmed-milk powder, meat-bone-meal or fishmeal and oilcakes. Depending on the place of origin some of these food components are good selenium sources (Lin 1968, Oel 1968, Gis 1969). Especially fishmeal may show a high concentration of selenium, and from 1.2 to 2.5 ppm in the dry matter has been measured in samples from Northern Europe (Lin 1968, Gis 1969). The pasture species on the average probably contain sufficient selenium, while of the root crops only swedes and other Cruciferae species contain enough (table 3.13).

Thus, with a supplement of concentrated foodstuff it seems probable that the selenium supply to cattle should in most cases be sufficient. Sheep are often fed on poor pastures, and the supply of concentrated foodstuff may be small. Selenium deficiency in sheep may therefore occur. The situation for pigs is less clear, especially owing to uncertainty about the requirements of monogastrics.

The present tendency towards a decreasing use of imported concentrated foodstuffs may increase the occurrence of selenium deficiency in livestock (including poultry) as many of the concentrates contain more selenium than our forage plants.

4. SELENIUM ABSORPTION BY PLANTS FROM SELENIZED SOILS

As a measure against selenium deficiency in livestock it can be desirable to increase the concentration of selenium in the foodstuff. Therefore, in order to estimate the amount and the compound one should add to the soil in such a case, it is necessary to study the absorption by the plants. Results of some previous studies (Gra 1965, Dav 1966a, b, Wat 1967) were quoted in subsections 1.1.4 and 1.2.2.

For further elucidation of the absorption by the plants a series of experiments was made in the years 1966 to 1968. The following parameters were considered: plant species, soil, selenium compound, amount of selenium, and influence of other additions to the soil. Of the agricultural plants used as test plants, barley, red clover and white mustard were chosen in most of the experiments. For some purposes, however, radish was preferred because it absorbs large amounts of selenium, and because it has a conveniently short growth period.

For pot experiments the selenium compounds were labelled with Se-75, and the native selenium was not accounted for. In the field no use was made of radioactive tracers and selenium determinations were therefore made by neutron activation analysis. A description of the experimental techniques was given in section 2.6 and earlier (Bis 1969, Gis 1970b), and a description of the soils was given in table 3.2.

4.1. Plant Species

For studies of the extent to which the selenium concentration in the plant varies with species, stage of development and part of the plant, one series of plants was grown in pots and another in the field, in both cases on selenized soils.

The main conclusion of the experiments is that the plant species can, to a good approximation, be ordered according to their affinity towards selenium. A multitude of data bears this out. The order of concentration for ten species studied in a pot experiment on a selenized soil (Bis 1969, table 4) was almost the same as the order emerging from the analyses of plants grown in the field on non-selenized soils (this evidence was discussed in tables 3.10 and 3.11 of this work) and from field experiments with added selenate (Gis 1970b, table 5). Fig. 4.1 ranks some plant species according to selenium affinity and illustrates the differences between the species.

Not surprisingly, the selenium is not uniformly distributed within the plant. In the following some results on selenium distribution are given.

For plants with storage organs (turnip, potato, etc.) those parts normally have a lower concentration of selenium than the leaves irrespective of

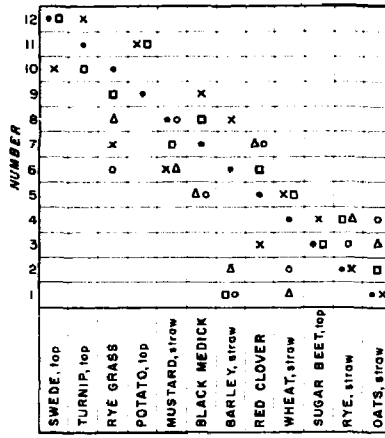

Fig. 4.1. Diagram showing the order of increasing selenium concentration for plants grown either in the field or in pots and with or without added selenium salts. The plants have been ordered (left to right) according to the concentrations found in field experiment No. II.

Key: • No Se added, field experiment No. II
x No Se added, field experiment No. III
□ Se^{+6} added, field experiment No. III
Δ Se^{+6} added, pot experiment No. 3
○ Se^{+4} added, pot experiment No. 3

whether selenium has been supplied or not, and irrespective of the oxidation state of any such supply (Gis 1970b). Similar results have been obtained for plants grown in pots on seleniferous or selenized soils (Fle 1962a, Ham 1964).

The distribution between grain and straw of cereals grown on selenized soils was in our experiments in favour of the grain, irrespective of whether the growth was in pots (tables 4.1 and 4.2) or in the field (Gis 1970b). The straw was favoured when no selenium was added to the soil (the field experiments in tables 3.10 and 3.11) and on Se-rich soils in other pot experiments (Joh 1961, Fle 1962a, Ham 1963a). It is common that intensive fertilization (as often in pot cultures) results in a distribution of a nutrient which is in favour of the grain. The distribution found in the pot cultures (tables 4.1 and 4.2) can be explained in this way, but for crops grown in the field (field exp. No. III), the addition of selenium alone increased the ratio (Gis 1970b, table 5). Not surprisingly mustard seeds seem in all cases to

Table 4.1

Plant yield and selenium concentration in plants grown on five soils supplied with 0.5 ppm Se as K_2SeO_4. Pot experiment No. 2.

	Crop	Soil No.**) 1	3	4	5	6
Yield of dry matter,*) g per harvest	Barley, green	49	39	31	47	62
	straw	24	37	42	39	54
	grain	27	42	52	39	40
	Red clover, 1st cut	31	31	26	18	49
	2nd cut	34	46	47	35	59
	White mustard, green	22	24	20	21	38
	straw	28	38	46	42	61
	seed	9	16	19	16	21
Se in dry matter, ppm	Barley, green	9.4	8.0	9.0	6.2	1.4
	straw	5.4	3.3	3.3	3.1	0.6
	grain	5.8	3.7	3.9	4.0	6.8
	Red clover, 1st cut	19.9	13.7	15.2	10.5	2.0
	2nd cut	19.3	13.8	11.9	10.3	1.5
	White mustard, green	25.7	26.2	32.0	27.4	4.2
	straw	8.0	6.1	5.6	5.0	2.5
	seed	13.6	16.8	15.2	11.6	1.9
Absorption quotient	Barley	0.075	0.058	0.062	0.056	0.039
	Red clover	0.127	0.105	0.095	0.055	0.019
	White mustard	0.090	0.113	0.119	0.098	0.037

*) Half of the crop in pots with barley and mustard was harvested just before flowering, the other half at maturity. For clover the whole crop was cut each time.

**) Soil data in table 3.2.

contain more selenium than the straw (tables 3.10, 3.11, 4.1, 4.2, Gis 1970b, table 5).

The concentration in the young plants was higher than in the straw, and in barley also higher than in the grain. This is in agreement with the behaviour of plants with respect to other elements (Stee 1965) and with data for indicator plants (Mox 1950, Ros 1964). It is, however, in contrast with other results for selenium in cereals (Lin 1968) and in rye grass (Lane 1966a). A comparison of the total amount of selenium absorbed by the plants at flowering and at full development has been made in table 4.3. There is a clear difference between the ratios for selenate- and selenite-supplied plants. When selenite is used, the absorption in the last part of the growth period is of importance. This means that in the meantime an oxidation of the selenite in the soils may have taken place. When selenate is used, the uptake takes place predominantly during the early part of the growth period. The results point

Table 4.2

Plant yield and selenium concentration in plants grown on five soils supplied with 0.5 ppm Se as K_2SeO_3. Pot experiment No. 2.

	Crop	Soil No.**) 1	3	4	5	6
Yield of dry matter, g per harvest*)	Barley, green	44	37	32	39	62
	straw	33	40	44	44	50
	grain	35	44	52	40	41
	Red clover, 1st cut	48	31	26	18	52
	2nd cut	32	54	53	34	62
	White mustard, green	26	19	19	19	41
	straw	28	46	44	47	57
	seed	9	19	20	19	20
Se in dry matter, ppm	Barley, green	1.81	1.42	1.22	0.83	0.44
	straw	0.99	0.59	0.53	0.29	0.26
	grain	1.08	1.15	0.91	0.84	0.84
	Red clover, 1st cut	1.88	1.94	1.27	1.25	0.77
	2nd cut	1.58	1.61	1.17	0.69	0.51
	White mustard, green	3.21	3.16	3.13	1.84	0.58
	straw	2.43	1.25	1.27	1.26	0.47
	seed	4.27	3.22	2.91	3.63	1.31
Absorption quotient	Barley	0.015	0.013	0.011	0.008	0.007
	Red clover	0.014	0.015	0.010	0.005	0.007
	White mustard	0.019	0.018	0.017	0.01[6]	0.008

*) See footnote to table 4.1

**) Soil data in table 3.2

towards a small loss of selenium during the latter part of the growth period (gaseous selenium compounds?), but the experimental errors are too large to allow a firm conclusion.

Depending on the plant species the absorption quotient for selenate added to mineral soils (0.5 ppm added Se) varied from 0.05 to 0.13 (table 4.1). For selenite the corresponding figures were 0.005 to 0.02 (table 4.2). Addition of increasing amounts of selenate or selenite resulted for both salts in increasing absorption quotients (table 4.4). The absorption quotients for selenate are comparable with those for other elements not absorbed or precipitated in the soil such as NO_3^- and K^+, while the quotients for selenite are comparable with that for Mo and larger than those for Mn and Cu (Stee 1965).

The yield was not affected by the application of 0.5 ppm Se either as selenate or as selenite. Thus in pot experiment No. 2 the yields in pots without addition of selenium were identical to those with added selenium in tables 4.1 and 4.2. When, however, the selenate addition was increased to 2.5

Table 4.3

Total amount of selenium absorbed by plants harvested at full development divided by the total amount absorbed until flowering.*)

Plant species	Soil No.	mg Se in old plants / mg Se in young plants	
		Se^{+6} added	Se^{+4} added
Barley	1	0.6	0.9
	3	0.9	1.4
	4	1.2	1.8
	5	1.0	1.5
	6	4.9	1.7
Mustard	1	0.6	1.3
	3	0.8	2.0
	4	0.9	1.9
	5	0.7	3.6
	6	1.2	2.2

*) Pot experiment No. 2. The soils were supplied with 0.5 ppm Se as K_2SeO_4 or K_2SeO_3. The experiment was carried out in triplicate, and half the crop of each pot was harvested just before flowering, the other half at maturity.

**) Soil data in table 3.2.

Table 4.4

Selenium concentration in plants and absorption quotients after addition of three different amounts of K_2SeO_4 or K_2SeO_3*).

Oxidn. state of added Se	Se added to the soil, ppm	Yield of dry matter, g/pot	Se in plant dry matter, ppm	Absorption quotient
+6	0.1	8.8	0.95	0.036
	0.5	8.5	9.9	0.073
	2.5	7.3	75	0.088
+4	0.1	8.7	0.28	0.011
	0.5	8.6	1.9	0.014
	2.5	8.3	13.3	0.020

*) Average for ten species all harvested once before flowering (pot experiment No. 3, soil No. 2). The species were: Oats, barley, rye, wheat, rye grass, red clover, lucerne, black medick, radish (leaves), and white mustard.

ppm Se, the yield decreased (table 4.4). The decrease was for ten species on an average 15 %, but it could be as much as 50 %. Of the ten species the ones most susceptible to intoxiation were three leguminosae, rye and wheat (Bis 1969). This is, however, of little interest in practice, because the plants become very toxic at this rate of application. Selenite in the same amount (2.5 ppm) did not affect the yield.

4.2. Soil

The influence of the soil on the absorption of selenium by the plant was measured in pot culture by means of six soils (tables 4.1, 4.2 and 4.5). Five of the soils are representative of common Danish soil types, while soil No. 6 is a muck soil (from the Lammefjord area). The test plants used on five of the soils (pot experiment No. 2) were barley, red clover and white mustard. These three test plants are taken to be representative also of other species with respect to their selenium content. In another experiment (pot experiment No. 5) a sixth soil (No. 2) was tested against soil No. 5 with mustard and radish (table 4.5). The difference in concentration obtained in mustard grown on soil No. 5 in experiment No. 2 (tables 4.1 and 4.2) and in experiment No. 5 is due to the growth conditions: outdoor and summer as opposed to greenhouse and winter.

Plants grown on the most sandy soils had the highest selenium concentrations, and those grown on the muck soil the lowest. Even in spite of the higher yields obtained on the muck soil, the absorption quotients for selenate are by far the lowest on this soil (table 4.1).

The clay content in the soil could seem to be decisive for the concentration in the plants. If the concentrations in the different species grown on soil No. 5 are used as a unit, relative concentrations obtained on the other soils can be calculated. The average for the three species of these relative concentrations are plotted for each soil (fig. 4.2) versus the clay

Table 4.5

Selenium in plants grown on soils No. 2 and 5 supplied with 0.5 ppm Se. Mustard (20 kg soil/pot) was harvested at early flowering, radish (2.5 kg soil/pot) after development of the hypocotyl. Pot experiment No. 5.

	Crop	K_2SeO_4 added		K_2SeO_3 added	
		Soil No. 2	Soil No. 5	Soil No. 2	Soil No. 5
Yield of dry matter, g/pot	White mustard	16.4	15.6	16.5	13.8
	Radish leaves	4.2	4.1	3.9	3.7
Se in dry matter, ppm	White mustard	159	118	4.0	2.1
	Radish leaves	142	109	3.2	2.4
Absorption quotient	White mustard	0.26	0.19	0.007	0.003
	Radish leaves	0.47	0.35	0.010	0.007

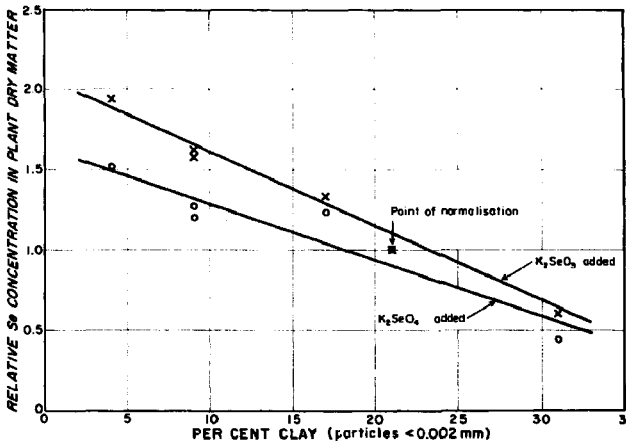

Fig. 4.2. Relationship between the clay content of a soil and the selenium concentration in plants (red clover, barley, mustard) grown on the soil. The selenium concentrations relative to those found in the same species grown on soil No. 5 are used. Pot experiments Nos. 2 and 5.

Table 4.6

Water-soluble selenium in five soils after harvest of two cuts of red clover. Pot experiment No. 2.

Soil No.	Oxidn. state of added Se (0.5 ppm)	Se in 2nd cut, ppm	Removed by 1st + 2nd cut, % of added	H_2O-soluble Se, % of added
1	+6	19.3	12.7	20
3		13.8	10.5	20
4		11.9	9.5	23
5		10.3	5.5	24
6		1.5	1.9	3
1	+4	1.58	1.4	12
3		1.61	1.5	18
4		1.17	1.0	20
5		0.69	0.5	21
6		0.51	0.7	13

content of the soil. One observes a relationship between the clay content of a soil and the selenium concentration in the plant. The relationship seems to exist for both selenate- and selenite-supplied soils. According to the figure, it may be assumed that the concentrations obtained in a certain crop grown on different Danish mineral soils should not vary by more than a factor of 2 or 3. For selenite application this relationship has been confirmed in a recent experiment using mixtures of a sandy and a loamy soil (Gis 1971a). It agrees also well with the result of Davies and Watkinson (Dav 1966b) that further led to the conclusion that the soil-selenium reactions are similar in all mineral soils.

A determination of the amount of water-soluble selenium (procedure 1, see 2.4.1) in the soils was made after the second harvest in pot experiment No. 2. The results are shown for the soils on which clover was grown (table 4.6). It is seen that the amount of soluble selenium is not correlated with the concentration found in the plants. Only in the muck soil supplied with selenate does the soluble amount correspond with the low concentration in the plants. Especially the similarity in the amount of water-soluble selenium in selenate- and selenite-supplied soils makes it clear that this figure is not a good measure of the availability of the added selenium.

4.3. Selenium Compound

The different availability to plants of potassium selenate and potassium selenite has already been illustrated in tables 4.1 and 4.2. A difference similar to the one seen there has been noted before both when plants were grown in water culture and in soil (Hur 1937b, Mox 1950).

The availability of a series of selenium compounds — among those, compounds of low solubility — were compared in a pot experiment. The solubilities of the compounds are given in table A.1 in the Appendix. The use of the fairly insoluble elemental selenium, though with a large surface, resulted in 0.02 to 0.8 ppm Se in the plant dry matter (table 4.7), which is more than obtained by Peterson and Butler (Pet 1966), but less than the effect found by Cary et al. (Cary 1969). Also in the field an application of elemental selenium caused an increase in the concentration in the plants (fig. 4.3). Although plants are able to absorb gaseous selenium compounds released from neighbouring plants (Broy 1966, Ash 1967) this is probably not the explanation here. Rather the increased concentration means that an oxidation of some of the selenium has occurred, in the pots already during the three months that passed between sowing and the first harvest. That this is possible has also been demonstrated by Geering et al. (Gee 1968). The absorption from a source of elemental selenium is, however, so small that it is probably of no practical interest.

Readily soluble and slightly soluble selenites showed no difference in availability (table 4.7, Wat 1967). It should be noted that the amounts of the

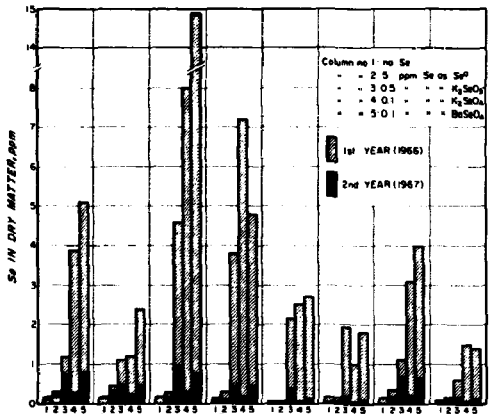

Fig. 4.3. Concentration of selenium in four plant species grown in the field. Selenium was added in the spring of 1966 in the amounts shown in the figure. The concentration in the soil is based on the upper 20 cm. Field experiment No. III (Gis 1970b).

slightly soluble selenites in table 4.7 in probably all cases exceeded those that could be dissolved in the water in the soil. For instance 0.3 ppm Se as $BaSeO_3$ is at least three times the soluble amount. If the dissolution is followed by an absorption or precipitation reaction, more may be dissolved and the influence of the solubility of the selenite compound thus eliminated. In pot culture the utilization of the selenites was 20 to 200 times that of the elemental selenium. About 1 % of the added selenite was removed by one crop.

Also for selenates the availability seems independent of the solubility; thus table 4.7 shows that the availability of $BaSeO_4$ is not smaller than that of K_2SeO_4 and $CuSeO_4$. In an experiment with equal concentrations (pot experiment No. 5, 0.5 ppm Se) about the same selenium concentrations were found in plants supplied with K_2SeO_4 and with $BaSeO_4$ (table 4 in Gis 1970b). The first year in the field the concentration in plants supplied with $BaSeO_4$ was also similar to that obtained after a K_2SeO_4 addition (fig. 4.3). The reason for this similarity may well be that Ba^{++} from dissolved $BaSeO_4$ is precipitated as the less soluble $BaSO_4$. The amount of readily available sulphate is 10 ppm (table 3.2) in both the soils used (Nos. 2 and 5). Thus there is sufficient sulphate even for a complete transformation of $BaSeO_4$ into $BaSO_4$ plus SeO_4^{--}.

Table 4.7

Absorption of selenium from different compounds labelled with Se-75. Pot expt. No. 4, soil No. 2 (Gis 1970b)

	Date of harvest	Yield of dry matter*)	ppm Se added to the soil									
			Se	SeO_2	K_2SeO_3	Na_2SeO_3	$BaSeO_3$	$FeSeO_3$	$CuSeO_3$	K_2SeO_4	$CuSeO_4$	$BaSeO_4$
			2.5	0.50	0.50	0.50	0.37	0.35	0.30	0.50	0.13	0.10
			ppm Se in dry matter									
Clover, 1st cut	7/7	34.4	0.05	1.43	1.55	1.33	0.87	1.08	0.70	36.7	21.9	22.6
Clover, 2nd cut	10/8	48.5	0.02	1.00	1.19	1.13	0.75	0.82	0.43	27.2	13.6	13.1
Barley, 1st cut	8/7	55.5	0.13	0.78	0.89	1.04	0.57	0.51	0.47	13.3	7.7	5.9
Barley, 2nd cut grain	17/8	30.0	<0.03	0.84	1.03	0.81	0.72	0.70	0.48	9.8	6.3	4.5
Barley, 2nd cut, straw	17/8	43.2	0.03	0.61	0.56	0.52	0.35	0.46	0.27	6.9	3.9	3.3
Mustard, 1st cut	24/6	31.2	0.83	1.80	1.96	1.80	1.00	1.11	0.53	49.2	24.6	26.3
Mustard, 2nd cut seed	16/8	17.8	0.42	2.18	1.91	1.60	1.06	1.32	0.98	25.6	15.4	15.4
Mustard, 2nd cut straw	16/8	50.9	0.05	0.66	0.63	0.48	0.34	0.38	0.22	8.9	5.2	5.1
			Absorption in per cent of added Se									
Clover			0.005	1.0	1.0	1.0	0.9	1.1	0.8	24	53	63
Barley			0.02	0.9	1.1	1.0	0.9	1.0	0.8	12	27	28
Mustard			0.07	1.2	1.3	1.1	0.9	1.1	0.7	24	61	48

*) Average yield in g per harvest. See footnote to table 4.1.

Table 4.8

Selenium in eight successive cuts of red clover. Different selenium compounds labelled with Se-75 were added to the soil. Pot experiment No. 4.

Cut No.	Date	Average yield of dry matter g/pot	Average ash content %	ppm Se in the dry matter after addition of:				
				Se° 2.5 ppm	K_2SeO_3 0.5 ppm	$BaSeO_3$ 0.37 ppm	K_2SeO_4 0.5 ppm	$BaSeO_4$ 0.1 ppm
1	7.7.66	33.1	12.4	0.05	1.55	0.87	36.7	22.6
2	10.8.66	47.4		0.02	1.19	0.75	27.2	13.1
3	15.10.66	28.5	7.5	0.07	0.92	0.51	14.6	6.2
4	16.1.67	28.0	10.0	0.05	0.63	0.35	7.7	3.3
5	7.4.67	28.9	8.1	0.06	0.42	0.24	4.0	1.8
6	30.6.67	45.3	7.8	n.d.*	0.27	0.16	2.0	0.83
7	3.8.67	31.4	7.1	0.04	0.30	0.20	1.7	0.78
8	28.8.67	18.8	7.3	0.05	0.58	0.17	1.4	0.52

*) Not detectable.

The absorption from the various selenium sources was followed over two years both in pot culture and in the field. Compounds with the same oxidation state behaved alike, and therefore not all the salts used in the pot experiment are included in table 4.8. After eight cuts (table 4.8) the concentration in the clover plants had decreased to 25 % of that in the first cut when selenites were the sources, and to only 4 % when selenates were the sources. In the pots no leaching could take place. In the field (fig. 4.3) the concentration in the selenite-supplied plants had decreased to 10 to 50 % the second year as compared with the first year. In the plants supplied with K_2SeO_4 the concentration was only 5 %, while in the plants supplied with $BaSeO_4$ it was 8 to 20 % as compared with the first year. Thus the effect the second year of 0.5 ppm Se as K_2SeO_3 was comparable with that of 0.1 ppm Se added as $BaSeO_4$. The smaller decrease in the effect of $BaSeO_4$ could be due to the low solubility, which implies that loss through leaching should be less than with K_2SeO_4 as long as not all $BaSeO_4$ has been transformed into $BaSO_4$.

4.4. Influence of Other Additions

The effect of varying amounts of sulphate, sulphite and ammonium nitrate upon the absorption of selenium was followed in a pot experiment. Besides, the effects of a large amount of organic material and of a change in the pH were measured.

As test plant was used radish (Raphanus sativus L.). After selenite addition the ratio between the concentrations in the leaves and the roots was fairly

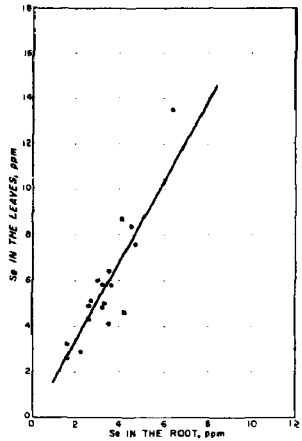

Fig. 4.4. Concentration of selenium in the leaves and roots (hypocotyl plus adherent roots) of radish grown on soils supplied with 0.7 ppm Se as selenite. Varying additions of sulphate, nitrogen and other amendments. Pot experiment No. 6.

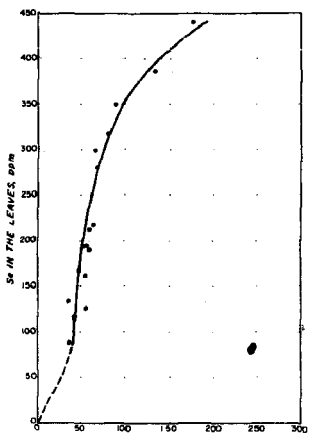

Fig. 4.5. Concentration of selenium in the leaves and roots (hypocotyl plus adherent roots) of radish grown on soils supplied with 0.7 ppm Se as selenate. Varying additions of sulphate, nitrogen and other amendments. Pot experiment No. 6. The extrapolation to the origin is made with the slope found in fig. 4.4.

constant 1.7 (fig. 4.4). After addition of selenate, the concentration in the leaves increased much more than that in the roots (fig. 4.5). From the change in the slope it is seen that the storage of selenium in the leaves is interrupted when the concentration exceeds 300 to 400 ppm. At this concentration in the leaves the absorption of sulphate is considerably increased as will be seen later (fig. 6.4).

The plants in fig. 4.5 showed no sign of intoxication, nor was the yield reduced (for reduction of yield at higher selenium concentrations (see table 6.2). Therefore, only the figures for the tops will be given in the tables. The first crop removed from 10 to 60 % of the added selenate and from 0.5 to 2 % of the added selenite.

Addition of increasing amounts of sulphate caused at first a decrease in the selenium concentration in the first crop (table 4.9) and in the absorption quotient (table 4.12) independently of whether selenate or selenite was the source. This is in contrast to the findings in a water culture experiment where sulphate only depressed the concentration when the source was selenate (Hur 1937b). In the second and the third crop the uptake from the selenate source at the lowest sulphate concentration was remarkably low as compared with that at the other sulphate concentrations. A pronounced decrease in selenium uptake occurs systematically from the second to the third crop grown on selenate-supplied soil (see also tables 4.10 and 4.11). This should at least partly be ascribed to the long interval between the last two crops, during which interval (about three months) the pots were not watered. The influence of the soil type was the same as seen previously, i.e. the highest selenium concentrations appeared in plants grown on the most sandy soil (No. 2).

The effect of 25 and 100 ppm S as sulphite on the selenium absorption was identical with that of the same amounts of sulphate whether the selenium source was selenate or selenite. This is presumably due to the fast oxidation in the soil of sulphite to sulphate.

The effect of increasing amounts of nitrogen on the selenium concentration in the plants is shown in table 4.10. The nitrogen was added as a constant amount of magnesium nitrate supplied with ammonium nitrate. In the first two crops a maximum concentration of selenium as well as of sulphur was obtained in the plants supplied with selenate at the moderate nitrogen additions. In the selenite-supplied plants the effect of nitrogen was less pronounced. The absorption quotients (table 4.12) followed the same pattern.

Organic matter, added as litter of leaves, depressed the concentration of selenium irrespective of whether the source was selenate or selenite (table 4.11), but the absorption quotients were fairly constant (table 4.12). The addition of 10 % litter (on a dry basis) increased the pH by 0.5 units.

Table 4.9

Influence of increasing amounts of sulphate on the uptake by radish of Se-75 labelled selenate and selenite. 0.7 ppm Se was added as potassium salts. The nitrogen addition corresponded to 150 ppm N. Sulphate was added as potassium and ammonium salts (the potassium addition was kept constant). Pot experiment No. 6.

Oxidn. state of Se	Soil No.	Crop No.	Yield of dry matter (leaves) g/6 pots				ppm Se in dry matter (leaves)				% S in dry matter (leaves)			
			ppm S added				ppm S added				ppm S added			
			5	30	100	250	5	30	100	250	5	30	100	250
+6	2	1	4.4	5.4	4.9	5.0	440	350	212	280	1.0	1.7	1.64	2.25
		2	6.2	5.0	5.9	5.3	98	180	176	160	0.81	1.7	1.81	2.06
		3	3.1	3.3	4.3	2.2	5	14	133	14	0.98	0.96	1.03	1.28
	5	1	5.2		5.0		386		114		1.17		1.22	
		2	6.5		5.3		54		92					
		3	3.3		3.3		0.7		29					
+4	2	1	4.7	6.0	5.7	6.3	13.5	8.7	5.8	7.6	0.72	0.84	1.03	1.16
		2	5.8	5.1	5.5	5.2	5.4	5.0	4.2	4.8	0.74	0.92	0.93	1.08
		3	3.2	3.1	3.1	3.2	2.1	2.4	2.1	1.4	0.84	0.93	1.09	1.08
	5	1	6.0		4.9		8.4		2.9		0.66		0.82	
		2	6.6		6.4		4.5		2.4					
		3	3.0		3.1		3.0		2.0					

*) Crop No. 1 was sown on Dec. 17, crop No. 2 on Jan. 25, and crop No. 3 on May 18.

Table 4.10

Influence of increasing amounts of nitrogen on the uptake by radish of Se-75-labelled selenate and selenite. 0.7 ppm Se was added as potassium salts. The sulphate addition corresponded to 100 ppm SO_4-S. Nitrogen was added as magnesium nitrate supplied with increasing amounts of ammonium nitrate. Pot experiment No. 6, soil No. 2.

Oxidn. state of Se	Crop No. *)	Yield of dry matter (leaves) g/6 pots					ppm Se in dry matter (leaves)					% S in dry matter (leaves)				
		ppm N added					ppm N added					ppm N added				
		25	75	150	225	300	25	75	150	225	300	25	75	150	225	300
+6	1	3.3	4.5	4.9	5.6	5.4	167	300	212	194	161	1.53	2.17	1.64	1.44	1.32
	2	1.4	2.3	5.9	8.5	7.1	88	135	176	155	121	1.22	1.54	1.81	1.58	1.62
	3	2.2	2.7	4.3	4.4	9.6	52	39	33	28	47					
+4	1	3.7	4.7	5.7	4.9	5.3	4.6	5.9	5.8	5.0	4.4	0.78	1.00	1.03	0.95	0.92
	2	1.4	2.2	5.5	8.5	9.6	2.7	3.0	4.2	4.2	3.8	0.74		0.93		1.02
	3	2.1	2.4	3.1	4.4	7.8	1.8	1.7	2.1	2.1	1.9					

*) For dates of sowing, see table 4.9.

Table 4.11

Influence of organic matter and of increased pH on the uptake by radish of Se-75-labelled selenate and selenite. 0.7 ppm Se was added as potassium salts. The sulphur and nitrogen additions corresponded to 100 ppm SO_4-S and 150 ppm N respectively. Decomposed leaves were used as organic material. The amounts used were on a dry basis. The pH was increased from 4.6 to 7.0 (in KCl) by means of 0.5 % $CaCO_3$. Pot experiment No. 6, soil No. 2.

Oxidn. state of Se	Crop No. *)	Yield of dry matter (leaves) g/6 pots				ppm Se in dry matter (leaves)				% S in dry matter (leaves)			
		No addition	2 % org. matter	10 % org. matter	$CaCO_3$	No addition	2 % org. matter	10 % org. matter	$CaCO_3$	No addition	2 % org. matter	10 % org. matter	$CaCO_3$
+6	1	4.9	5.8	6.5	2.9	212	193	133	127	1.64	1.50	1.31	1.20
	2	5.9	5.5	6.1	6.8	176	138	148	155				
	3	4.3	3.4	5.5	5.0	33	34	31	53				
+4	1	5.7	5.4	5.9	3.6	5.8	5.1	3.2	4.1	1.03	1.01	0.92	0.87
	2	5.5	5.7	6.7	5.7	4.2	4.0	2.9	5.2				
	3	3.1	3.9	5.3	5.1	2.1	1.7	1.3	6.6				

*) For dates of sowing, see table 4.9.

Table 4.12

Absorption quotients for selenium as a function of the addition of sulphate, nitrogen and organic material (pot experiment No. 6, 1st crop, leaves only, soil No. 2).

Addition	Concentration	Absorption quotient	
		Se^{+6}	Se^{+4}
S	5 ppm	0.46	0.015
	30 ppm	0.45	0.012
	100 ppm	0.25	0.008
	250 ppm	0.33	0.011
N	25 ppm	0.13	0.004
	75 ppm	0.32	0.007
	150 ppm	0.25	0.008
	225 ppm	0.26	0.006
	300 ppm	0.21	0.006
O.M.	0%	0.25	0.008
	2%	0.27	0.007
	10%	0.21	0.005

An increase of the pH from 4.6 to 7.0 (measured in KCl) by means of $CaCO_3$ decreased the yield as well as the selenium concentration in the first crop (table 4.11). A similar effect upon the absorption was found for plants grown on soils with different pH values (tables 4.1 and 4.2). But owing to the considerable reduction of the yield of the first crop, the lower concentration may as well be a result of the poor growth conditions for radish on an alkaline soil. The sulphur concentration was affected similarly.

It is noted that in the selenate-supplied plants the concentrations of selenium and sulphur follow each other as long as the supply of both elements is constant (tables 4.10 and 4.11). The interrelationships of selenium and sulphur will be considered further in chapter 6.

4.5. Oxidation State of Selenium in Radish

The oxidation states of selenium in plants were determined in aqueous extracts of radish leaves and roots. Radish was chosen because it is one of the species absorbing most selenium. The dried plants from pot experiment No. 6 were used. Data for this material are presented in figs. 4.4 and 4.5 and tables 4.9 to 4.12.

The percentage of soluble selenium was somewhat larger in the selenate-supplied plants as compared with the selenite-supplied ones (table 4.13). At least for the roots this may be due to reduction of selenium to the

elemental form as was seen after addition of 2.5 ppm Se as selenite to the soil. The lower percentage of water-soluble selenium in the roots as compared with the leaves can to some extent be ascribed to an inefficient washing of the voluminous pulp. Because of the treatments the concentration in the leaves varied from 130 to 440 ppm after selenate addition and from 3 to 14 ppm after selenite addition (tables 4.9 to 4.11). Nevertheless the percentage of water-soluble selenium was fairly constant. This was also found for the roots.

Organic selenium, selenate and selenite were found in all extracts. The distribution between the three fractions did not depend systematically upon the sulphur and nitrogen additions to the soil. The plants supplied with selenite seem to have a larger part of the soluble selenium bound in organic compounds than the selenate-supplied plants.

Trelease and Beath (quoted in Ros 1964) also found with maize in water culture that an addition of selenate resulted in a higher percentage of water-soluble selenium in the plants than when the source was selenite. Still according to these authors, most organic selenium was found in extracts of selenite-supplied plants, just as in table 4.13. But no selenite was detected in the maize plants. Nor has selenite been found in most of the other investigations (Bea 1947, Ham 1963a, b, Ham 1964). Those investigations, where the less sensitive chemical determinations were used, have comprised many species among others radish (Ham 1964). Only Peterson and Butler, who used Se-75 as a tracer, found from 8 to 90 % selenite in extracts of various plant species (Pet 1962). In the present experiments 35 to 50 % selenite was found in the extracts of radish (table 4.13). It is not probable that this selenite should derive from reduction of selenate in the extract. A control precipitation showed that after addition of Se-75-selenate, no radioactive selenite was found in extracts of non-labelled radish. On the other

Table 4.13

Chemical form of the water-soluble selenium in radish. Average of ten treatments ± standard deviation. Pot experiment No. 6.

Oxidn. state of Se added to the soil	Plant part	Se in dry matter, ppm	Water-soluble Se, %	Distribution of the forms of the soluble Se		
				Org. Se	Se^{+4}	Se^{+6} **)
+6	Leaves	244	78 ± 7	5 ± 1	50 ± 0	45 ± 1
	Root *)	72	48 ± 7	19 ± 3	38 ± 6	43 ± 7
+4	Leaves	64	65 ± 3	19 ± 2	39 ± 6	42 ± 6
	Root *)	36	31 ± 2	42 ± 8	35 ± 8	23 ± 11

*) By root is understood hypocotyl plus adherent roots.

**) Se^{+6} is obtained as the difference between "soluble Se" and "org. Se + Se^{+4}".

hand, a hydrolysis of organic selenium cannot be completely ruled out though changes of the extraction time did not affect the distribution between the three fractions. The conclusion seems to be that selenite in all probability does occur in plants in much larger concentrations than generally believed till now.

4.6. Discussion

Selenates as well as selenites may be of use for artificial increase of the selenium concentration in crops. Elemental selenium is oxidized so slowly that it seems without practical interest. For selenates and selenites it was found that over a shorter period the oxidation state is the decisive factor for the absorption by the plants, while the solubility was of no importance (table 4.7). Over a longer period the low solubility of $BaSeO_4$ seemed to make this salt preferable to the readily soluble selenates for field use as long as only the resulting concentration in the plants is regarded.

However, none of the salts investigated in the present work fulfil the requirements made of an ideal compound: A constant supply either for one year or for a much longer period. The selenates give a high initial supply which already during the first growth season declines fast. This is a disadvantage especially for green crops harvested several times during the summer. On the other hand the selenates have the advantage of a relatively high absorption quotient. The selenites offer a more moderate initial supply, and the fall in concentration between early and late cuts of green crops is smaller than after application of a selenate. An obvious disadvantage of the selenites is the possible accumulation in the soil (see chapter 5). Besides, the absorption quotients are small, and an addition giving an adequate concentration one summer will often be too small the next, especially on acid soils.

The selenium concentration in plants grown in pots on soils supplied with selenate was 2 to 50 times as high as when the same amount of selenite-Se was added (tables 4.1, 4.2, 4.4, and 4.5). In the field 0.1 ppm selenate-Se resulted in plants with a concentration from the same to three times that obtained with 0.5 ppm selenite-Se (fig. 4.3). A tendency was found towards an increasing absorption quotient at increasing additions of either selenate or selenite (table 4.4).

In pot cultures an addition of 0.1 ppm Se as selenite resulted in about 0.3 ppm Se in the dried plants, and 0.1 ppm Se as selenate resulted in a few ppm in the plants. In the field an amount of selenite equivalent to 0.5 ppm Se (calculated on the upper 20 cm, i.e. 1250 g Se/ha) gave concentrations of less than 5 ppm in the plants, while 0.1 ppm as selenate (250 g Se/ha) gave on an average 5 ppm. If a concentration of 0.2 to 0.4 ppm Se in the plant dry matter is the aim, the addition should be about one tenth of these amounts. In New Zealand 70 g selenite-Se per ha added as a top dressing has been

recommended (Gra 1965, Dav 1966a). This corresponds to about 0.03 ppm Se in a 20-cm layer of the soil. In a later field experiment an addition of 100 g selenite-Se per ha resulted on the average in an increase of only 0.02 to 0.06 ppm Se in the crop (Gis 1971).

The result of a given addition will, however, in each case depend on the growth conditions and the plant species. The variation with some of these factors was discussed in the preceding sections. A very important soil property is the clay content with which the concentration in the plants seems to vary inversely. The amounts of nutrients in the soil will also affect the concentration. Thus varied nitrogen and sulphur additions meant another factor of 2 in the concentration range. The influence of the amount of precipitation has not been studied with selenized soils. But in the field the selenium concentration in a clover-grass mixture grown on a non-selenized soil did not seem to depend upon the water supply (chapter 3.2.3). The most influential factor of all is the plant species. In the common agricultural crops the concentration may vary by one order of magnitude if selenate is used, and by a factor of 5 if selenite is used.

5. CHEMICAL CHANGES OF SELENIUM COMPOUNDS IN SOILS

Selenium compounds added to soils may undergo a variety of chemical reactions which may result in a change in the availability to plants. Oxidation or reduction processes are frequently of importance as are precipitation reactions such as the formation of slightly soluble iron selenites. Further microbiological activity may lead to reduction and incorporation of Se in organic compounds, but there is also evidence (Shr 1967) of the existence of micro-organisms that can oxidize selenite to selenate.

In connection with some of the pot experiments an attempt was made to determine the amounts of selenate, selenite and organic selenium in aqueous extracts of the soils after cropping. However, only a minor part of the soil selenium was extractable with a selenite solution, and the distribution among the oxidation states could only be roughly determined owing to a low level of radioactivity. Therefore, selenized soil samples were also stored in vessels for six months, and the amounts of different selenium compounds were then determined. From two of these soils a release of volatilized selenium was measured.

Leaching can be another factor decisive for the amount of plant-available selenium present some time after the addition. Leaching was followed in the field, and the result will be discussed briefly here.

5.1. Preparation of Selenized Soil Samples

Five soils of the types previously used in pot experiments (see table 3.2) were in 1968 taken for selenization and later extraction. Samples of 100 g were mixed with the Se-75-labelled selenium compound and any other addition. The moisture content was brought to 15 %, or more when necessary, to obtain a crumbed structure. The samples were stored in darkness at room temperature for six to eight months. Then duplicates of each sample were extracted in accordance with the methods described in subsections 2.4.1 and 2.4.2. The experiment comprised the series listed below. Selenium was added as K_2SeO_3 and as K_2SeO_4 respectively in all three series. Besides, series A was repeated with $BaSeO_4$.

Series A: Soils Nos. 1, 2, 4, 5, and 6; addition of 0.1 ppm Se.

Series B: Soil No. 5; addition of 0.01, 0.1, 1, and 10 ppm Se.

Series C: Soil No. 1; the pH was adjusted by means of calcium carbonate and calcium oxide to cover a pH range from 3.5 to about 9 (measured in KCl); 0.1 ppm Se was added.

5.2. Results

The oxidation states of the added selenium were determined by two procedures. In procedure I the analysis was made on an aqueous extract (subsection 2.4.1). A subsequent extraction of the soil with strong sulphuric acid should, according to Williams and Byers (Will 1936), bring most of the remaining selenium into solution, including a not well-defined part occluded in iron oxides. Procedure II was that of Cary et al. (Cary 1967) and could only be applied to the soils in the experimental series A to C. By means of a sequence of extractants (see fig. 2.13) the chemical character of the compounds was determined. It was assumed that selenium soluble in K_2SO_4 mainly included selenates, but also some selenites and soluble organic compounds. $NaHSeO_3$-soluble selenium included isotopically exchangeable selenites. Ammonia-soluble selenium included absorbed and occluded selenites and selenates and organic selenium, probably protein-bound seleno amino acids. The latter group can be precipitated with HCl. Extraction with strong hydrochloric acid should dissolve some selenites and transform iron selenides into elemental selenium. This is dissolved in HNO_3 together with any other elemental selenium.

Analysis made after four, six and eight months of storage showed that after four to six months the fractions were fairly constant. Only the readily soluble selenium showed a halving in this period (on the average from 19 % to 10 %), and the ammonia-soluble part increased correspondingly (from 26 % to 34 % of that added).

The conclusions are based upon extractions made six to eight months after the addition of selenium to the soils, and are not sensitive to chemical changes during these two months.

5.2.1. Soil Type

The chemical form of selenium in five soils supplied with selenate or selenite and stored for six months are shown in figs. 5.1 and 5.2. As could be expected, the amount extracted with a selenite solution (procedure I, first column in the upper part of the two figures) corresponds very closely to the amount of selenium present as soluble Se plus isotopically exchangeable selenite (the first two columns in the lower part of the figures). This was also found in the other experimental series. The other fractions in the two extraction procedures are not comparable. A precipitation of selenium from the sulphuric acid extract (procedure I) showed that part of this selenium was present as selenite, but the form of the remainder could not be determined. All in all, the extraction procedure II which is the one proposed by Cary et al. (Cary 1967) seems to be the most informative. The selective precipitation of selenate in procedure I is, however, a valuable confirmation of the presence of this oxidation state.

With the concepts of Cary et al. (Cary 1967) it is seen that whether selenium is added as selenate or as selenite, the following compounds are present in all five soils six months later: Selenate, selenite, organic selenium, and either elemental selenium or selenides or both. The amounts of selenate and selenite are larger when the respective salt has been added to the soil. Two fractions are not shown in figs. 5.1 and 5.2. These are the selenites dissolved in 6N HCl and the residue in the soil after the extractions. The former constituted from 2 to 6 % of that added, and the non-extractable selenium was from 4 to 11 % of that added.

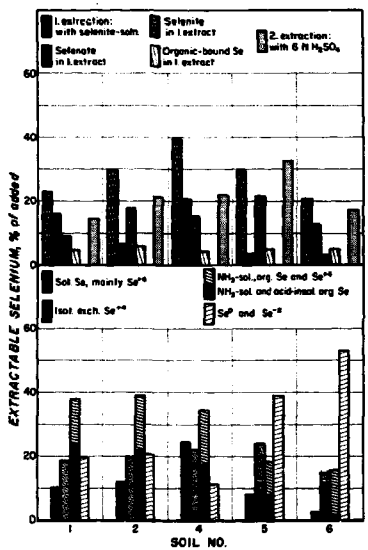

Fig. 5.1. Chemical forms of selenium in five soils to which 0.1 ppm Se as K_2SeO_4 had been added (series A). After the addition the soils were stored in vessels for six months. The upper part of the figure shows the results of extraction with a selenite solution (50 ppm Se) followed by extraction with hot sulphuric acid (procedure I). Below, the results are shown of a sequence of extractions with various extractants (procedure II). The two columns with identical hatch patterns in both parts of the figure should represent nearly the same fractions. The clay content and the pH of the soils are both increasing when going from the left to the right of the figure, while on the average the absorption quotients and the concentrations in red clover, white mustard, and barley decrease from soil No. 1 to soil No. 5 (tables 4.1 and 4.2).

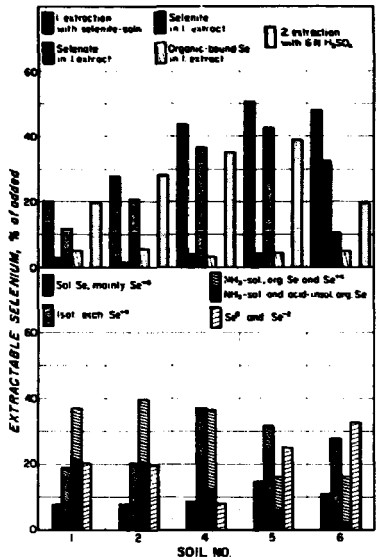

Fig. 5.2. Chemical forms of selenium in five soils to which 0.1 ppm Se as selenite had been added (series A). Otherwise the conditions are identical to those in fig. 5.1.

For samples with added $BaSeO_4$ the fractions showed the same pattern as for K_2SeO_4, and no influence from the difference in solubility was observed.

None of the fractions in figs. 5.1 and 5.2 seems to provide a measure of the plant-available selenium. Thus in no case was a difference found that would reflect the difference in uptake between selenate and selenite supplied plants. The amount of the extractable selenium was increasing with increasing pH and clay content of the soil when selenite was supplied (first column in upper part of fig. 5.2), whereas the absorption by plants was decreasing (table 4.2). The only fraction which seemingly may be related to the availability is the ammonia-soluble, but acid-insoluble selenium (lower part of figs. 5.1 and 5.2). According to Cary et al. (Cary 1967) this fraction consists mainly of protein-bound seleno amino acids. Though plants are able to absorb some soluble organic selenium compounds, there is hardly any doubt that readily soluble inorganic selenium compounds also are important for the supply of the plants.

In a pot experiment (No. 4) the amounts of Se^{+6}, Se^{+4} and organic Se in water extracts of the soil were determined after six months of cropping. The

amount of radioactivity was rather small at that time and procedure I was therefore used. As extractant was used water instead of a selenite solution as in figs. 5.1 and 5.2. From this soil (No. 2) water extracts about 80 % of the amount extractable with a selenite solution. The results from the cropped soil (table 5.1) are in good agreement with the results for the same soil stored in vessels for a similar length of time (the upper parts of figs. 5.1 and 5.2). It is to be noted that after addition of elemental selenium a little soluble selenium was found in some of the soil samples after cropping.

Table 5.1

Water-soluble selenium in soil No. 2 six months after the addition of respectively 5 ppm Se as elemental selenium, 0.3 to 0.5 ppm Se as selenites (six salts), and 0.1 to 0.5 ppm Se as selenates (four salts). The soluble amount did not vary much with the preceding crop, and the figures given are average values for soils cropped with barley, red clover and white mustard. (Pot experiment No. 4)

Selenium added as	Water-soluble Se, % of that added	Distribution of the soluble Se		
		% Se^{+6}	% Se^{+4}	% org. Se
Se^{0}	2 *)		0.8	
Se^{+4}	20	1.6	15	3.4**)
Se^{+6}	38	10***)	21	8**)

*) No detectable soluble Se in soil cropped with barley.

**) No detectable organic Se in soil cropped with barley.

***) No detectable Se^{+6} in soil cropped with clover.

5.2.2. Concentration of Selenium

The amount of selenium added to the soil seems to influence the distribution of selenium between the fractions (table 5.2). As far as can be seen from these data 0.1 ppm in the soil should mean a faster change of the original compound than at other concentrations, and the fraction containing Se° + Se^{-2} was consequently the largest. At 10 ppm the fraction of soluble Se contained about half the addition, and the most reduced fraction contained only 10 %. In all fractions the quantity of selenium increased with the concentration in the soil.

Table 5.2

Influence of the amount of added selenium on the chemical forms eight months later (series B)

Se added	Conc. in the soil	Selenium fractions, % of added				
		Soluble Se	Isot. exch. Se^{+4}	NH_3-sol. org. Se + Se^{+4}	NH_3-sol., but acid-insol. org. Se	Se^0 + Se^{-2}
K_2SeO_4	0.01	19	21	22	6	26
	0.1*)	8	24	18	8	39
	1	24	22	17	4	26
	10	54	20	8	1.5	11
K_2SeO_3	0.01	31	30	16	2	15
	0.1*)	15	31	16	6	25
	1	29	29	14	3	18
	10	41	30	12	1	10

*) These samples were analysed forty days earlier, which implies that the percentage of soluble Se might be too high, and the precentage of ammonia-soluble Se might be too low as compared with the results for the other concentrations.

5.2.3. pH of the Soil

pH is one of the soil properties which must influence the processes that the added selenium compound undergoes. Hence the most acid soil was adjusted to pH values from 3.5 (the original pH) to 9 as measured in M KCl. These values are up to 0.9 units below the values that would be found in an aqueous suspension (Hen 1969).

Only a slight reduction of added selenate was found in the pH range 4.5 to 6, and correspondingly the concentrations in the other fractions were low (fig. 5.3). At the highest pH values an increase of the fraction containing elemental selenium and selenides was seen. Both these results are in agreement with those for a series of soils in fig. 5.1. In soils with added selenite the fractions were more or less constant at pH values below 7. At higher pH the selenate fraction increased and the other fractions were similar to those in the soil with added selenate. The pH in the soils after the six months of storage did in most cases not differ by more than 0.2 units from the original. Only the samples adjusted to pH 9 had changed more, and the pH was now 7.6 to 7.9.

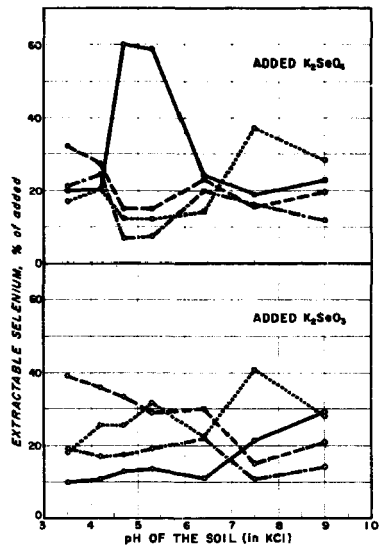

Fig. 5.3. The amount of selenium made soluble by a series of extractions (procedure II) is shown as a function of the soil pH (series C). The extractions were made six months after adjustment of the soil at the different pH values and addition of the selenium compounds. The pH values used are those measured at the start of the experiment.

——— Soluble Se, mainly selenate
—·—·— Isotopically exchangeable selenite
— — — Ammonia-soluble, organic Se and selenites
----- Elemental Se and heavy metal selenides

5.2.4. Volatile Selenium

The formation of gaseous selenium compounds in selenized soils was demonstrated. Samples of 15 g selenized with $BaSeO_4$ were used (series A). After four months of storage, moist air was led through the samples and then through two absorbers of granulated active carbon. In the second absorber no activity was detected, but in the first one Se-75 was detectable after twenty-four hours in two out of three soils (table 5.3). The values are to be considered lower limits as some of the volatile selenium formed during storage certain-

Table 5.3

Volatile selenium in soils four months after the addition of 0.1 ppm Se as $BaSeO_4$

Soil	% of added
No. 1, Tarm	~ 0.07
No. 5, Risø	~ 0.04
No. 6, Lammefjord	not detectable

ly will have escaped from the non-air-tight vessels and during the transfer of the soil to the aeration system. A larger release of gaseous selenium compounds than reported here has been found from soils aerated for three weeks immediately after selenite addition (Abu 1968).

5.2.5. Selenium in a Soil Profile

As long as a selenium compound remains in a soluble form, leaching can cause a loss. In the present investigation leaching could, however, only take place in the field. In one of these experiments selenium was determined in the soil profile six months and eighteen months after the addition. Four selenium compounds had been added (field experiment No. III). From the upper 25 cm five samples were taken from each plot of 2 m^2, from the deeper layers only three samples per plot.

The amounts of total as well as of water-soluble selenium were determined by means of neutron activation analysis as no selenium tracer had been used. The extraction was carried out at room temperature with a soil: solution ratio of 1: 2.5 and shaking overnight (see 2.4.1). This extraction is only half as efficient (table 2.11) as the hot extraction used in other cases.

As a whole there is reasonable agreement between the amount of selenium found in the core and the sum of native selenium plus added selenium (table 5.4). Selenium removed by the crop during the two years was about 0.8 $\mu g/cm^2$ from the selenate sources and 0.5 $\mu g/cm^2$ from the selenite source. This means that these amounts are without importance in this connection. It is seen that not only the elemental selenium, but also the selenite remained in the upper 25 cm of the soil. The selenate addition was so small as compared with the original content that no information can be drawn from these figures. One to 2 % of the native selenium was present in a water-soluble form. After the selenite addition this figure was increased to 7 % in the upper 10 cm the first autumn. This means that 10 to 20 % of the selenite had remained in a soluble form. The second year no difference was seen in the solubility of the native and the added selenium. K_2SeO_4 did not influence the amount of soluble selenium at all. The addition of $BaSeO_4$, however, increased the

Table 5.4

tal amount of selenium in soil profiles (field experiment No. III). Selenium compounds had been ded in the spring, and the soil samples were taken in October of the same year and the next year. ch years the crop was white mustard. The figures (in μg) are calculated for a soil column with 1 cm^2 cross section.

Year	Depth cm	Native Se	Se^0 added 125 μg/cm^2	K_2SeO_3 added 12.5 μg/cm^2	K_2SeO_4 added 2.5 μg/cm^2	$BaSeO_4$ added 2.5 μg/cm^2
1st	0-25	11	105	27	20	11
	25-50	7	16	9	11	9
	50-75	6	12	8	4	4
	0-75, total	24	133	44	35	24
	0-75, theor.		147	34	24	24
2nd	0-25	9	168	30	9	9
	25-50	8	18	9	8	6
	50-75	3	7	7	6	5
	0-75, total	20	193	46	23	20
	0-75, theor.		147	34	24	24

amount of soluble selenium in the first 25-cm layer of soil both years. Ten and 8 % were soluble, respectively. As the addition is rather small compared with the native selenium, this means that a substantial part of the added selenium remained soluble. The volume of water used for the extraction was just large enough to bring all the selenium — if present as $BaSeO_4$ — into solution. The result agrees with the higher concentrations the second year in plants supplied with $BaSeO_4$ as compared with those supplied with K_2SeO_4 (fig. 4.3).

5.3. Discussion

The redox potential in soils is to a first approximation lower the higher the pH, and the potential decreases when a soil is adjusted to a higher pH (Blac 1968). In soils with potentials ranging from −0.2 to + 1.0 volts and with pH values from 5 to 9, Se^0, Se^{+4} and Se^{+6} are the stable oxidation states (fig. A.1). But even if the potentials were known for the soils used here, the rates of the processes and the final distribution on the oxidation states cannot be predicted.

From the results it is clear that both reduction (of added selenate and selenite compounds) and oxidation (of added selenite) occur in all five soils. Selenite is refound not only as exchangeable selenite, but also (in order of decreasing amounts) as ammonia-soluble selenites, as nitric acid-soluble compounds, as ammonia-soluble, but acid-insoluble Se, and as selenate. The

restricted movement of selenium added as selenite is in agreement with this, and the relative importance of the various fractions are comparable with those found by Cary et al. (Cary 1967). Selenate is refound as exchangeable selenites, as ammonia-soluble selenites, as ammonia-soluble, but acid-insoluble Se, as compounds soluble in nitric acid, and as selenate. The distribution between the fractions depends, however, somewhat upon the concentration. It is thus clear that an accumulation of selenium in the soil is a possibility both when selenites and when selenates are applied. Furthermore, at least after application of selenate a small amount was released in a volatile form.

The main difference between selenate- and selenite-supplied soils was the occurrence of a maximum in the selenate fraction (in selenate-supplied soils) at pH values about 5 to 7 (figs. 5.1 and 5.3).

Neither of the analytical methods used in this series of experiments seems to provide a tool for predicting selenium uptake by plants and for assessing the selenium reservoir. This is forcefully borne out if one compares the very similar patterns for soil No. 2 in figs. 5.1 and 5.2 with the strikingly different uptake by clover at a similar time after selenate and selenite application (4th cut in table 4.8).

6. EFFECT OF SELENIUM UPON THE SULPHUR CONCENTRATION IN PLANTS

It is well known (see 1.2.2) that the addition of sulphate to growth media containing selenate may lead to a reduced selenium concentration in the plants. Only in a few investigations (cited in 1.2.1), however, have the concentrations of both elements been determined concurrently, and the body of information dealing with the effect of selenium compounds upon the uptake of sulphur is comparatively small. In one case (Hur 1938) an increase of the sulphur concentration was measured for intact plants when selenium was added to the nutrient solution. Other works (cited in 1.2.3) deal only with isolated plant organs. From these works it would seem that the addition of selenate mostly leads to a decrease in the absorption of sulphate.

In the present work data showing the concentrations of both sulphur and selenium in several plant species grown on non-selenized soils (tables 3.10 and 3.12) and on soils supplied with a constant amount of selenate or selenite (tables 4.9 to 4.11) have already been presented. In the following further data are given for a few species grown on soils supplied with a constant amount of selenium. Besides, results are presented showing the effect of increasing amounts of selenate and selenite additions upon the sulphur concentration. The influence of the two ions upon the uptake of sulphur and its metabolism was followed by means of the concentration of water-soluble sulphate and organic sulphur in the plants.

6.1. Sulphur Concentration in Plants Supplied with a Constant Amount of Selenium

The uptake of sulphur from different soils was compared in three species grown without and with a supply of selenate. Among the five soils used, four were mineral soils containing 5-15 ppm of readily available SO_4-S, while the fifth (soil No. 6, a muck soil) contained 270 ppm (table 3.2). Together with the fertilizers, 7 ppm SO_4-S was added. The sulphur content of the plants not supplied with selenate shows, however, no influence from the amount of available sulphate in the soils (table 6.1). In plants grown on the soils supplied with selenate an increase was found in the sulphur concentration as compared with the plants grown without a supply of selenate. Further, this increase was to some extent related to the absorption of selenium from the soils (table 6.1). For instance with clover as the test plant, the increase in sulphur concentration was a linear function of the selenium concentration.

Table 6.1

Sulphur and selenium concentration in three plant species harvested just before flowering. The plants were grown on five soils with no addition or an addition corresponding to 0.5 ppm Se as selenate. Pot experiment No. 2.

Soil No.	Clover			Barley			Mustard		
	No Se	+ Se^{+6}		No Se	+ Se^{+6}		No Se	+ Se^{+6}	
	ppm S	ppm S	ppm Se	ppm S	ppm S	ppm Se	ppm S	ppm S	ppm Se
1	2300	5300	19.9	1820	2300	9.4	3870	5600	25.7
3	2430	4240	13.7	1800	2300	8.0	5800	7600	26.2
4	2300	4860	15.2	2160	2640	9.0	7430	8820	32.0
5	2200	3340	10.5	1800	2050	6.2	5600	8920	27.4
6	2270	2700	2.0	1390	1570	1.4	7200	5800	4.2

Another example of the relationship between sulphur and selenium concentrations was seen in successive cuts of clover; in fig. 6.1 the corresponding concentrations are plotted against each other (the Se data

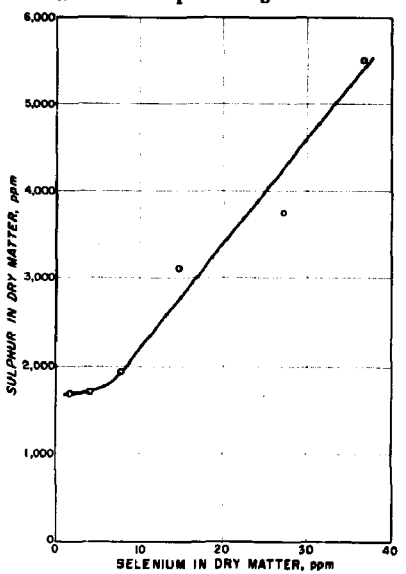

Fig. 6.1. Relationship between selenium and sulphur in successive cuts of red clover (pot experiment No. 4, selenate added).

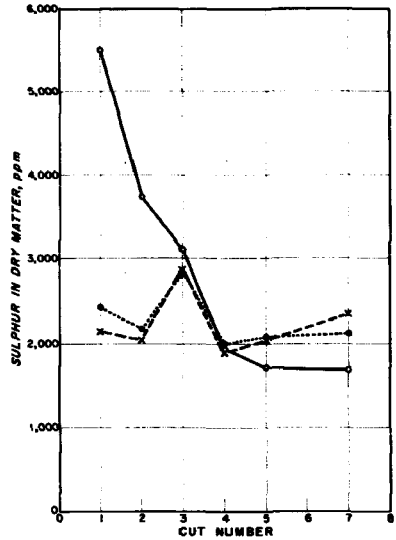

Fig. 6.2. The variation in sulphur concentration in successive cuts of red clover. (Pot experiment No. 4)

•----•	No addition of Se
x — — x	K_2SeO_3 addition, 0.5 ppm Se
o——o	K_2SeO_4 addition, 0.5 ppm Se

were given in table 4.8 under K_2SeO_4 addition and the S data are found in fig. 6.2). A linear relationship seems to exist over a broad concentration range.

In the field (field experiment No. III) an addition corresponding to 0.1 ppm Se in the plough layer resulted mostly in a concentration in the plants of 1 to 10 ppm Se. At these concentrations, the increase in the sulphur did not exceed 20 %.

The addition of selenite (0.5 ppm Se) to different soils had no effect on the sulphur content of the plants neither in pot nor in field experiments. Also in successive cuts of red clover a fairly constant sulphur concentration was found when the soil was supplied with selenite (fig. 6.2).

6.2. Sulphur Concentration in Plants Supplied with Varying Amounts of Selenium

The sulphur absorption as a function of the selenium supply was followed in experiments where sulphate labelled with S-35 was added to the soil (pot

experiments Nos. 7, 8 and 9). The technique has been described in section 2.6. By chemical determination of the sulphur in a number of the plant samples it was ascertained that the dilution of the added sulphate with soil-sulphur was negligible. The selenium determinations were in these experiments made by atomic absorption analysis and were therefore not very precise. Radish (Raphanus sativus L.) was mostly used as test plant, except for one case in which perennial rye grass was used.

6.2.1. Varied Selenate Addition

When the selenate addition was increased from 1 to 10 ppm Se, while the sulphate addition was kept constant, the selenium concentration in radish leaves increased from 250 to 2500 ppm (fig. 6.3). Probably on account of the small pot size used in this experiment, the concentrations corresponding to 1 and 2.5 ppm Se in the soil were higher than those obtained in another pot experiment with radish (pot experiment No. 3, Bis 1969). In the roots the concentration increased only by a factor of 2 when a change was made from

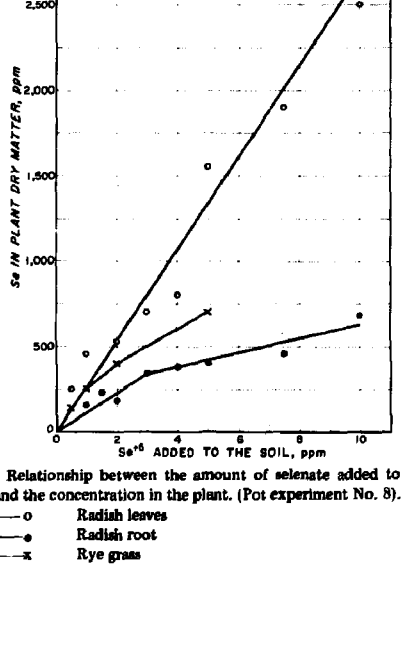

Fig. 6.3. Relationship between the amount of selenate added to the soil (No. 2) and the concentration in the plant. (Pot experiment No. 8).

o——o Radish leaves
•——• Radish root
x——x Rye grass

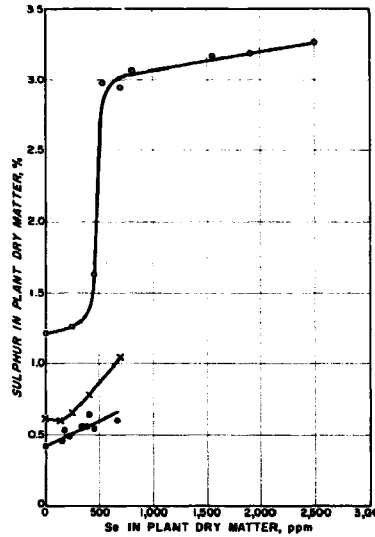

Fig. 6.4. The sulphur concentration as a function of the selenium concentration in plants grown on soil No. 2 containing 0 to 10 ppm selenate-Se (Pot experiment No. 8).

o——o Radish leaves
●——● Radish root
x——x Rye grass

1 to 10 ppm Se in the soil (fig. 6.3). Corresponding sulphur and selenium concentrations in the plants are shown in fig. 6.4. Also with respect to sulphur radish leaves showed the largest response. Thus the increase in selenium concentration from 200 to 600 ppm (1 to 2 ppm Se^{+6} in the soil) resulted in an increase in the sulphur content from 1.2 to 3.0 %. In a previous experiment (pot experiment No. 7) the addition of 2 ppm Se^{+6} resulted in a similar increase of sulphur in radish leaves (from 1.1 % with no Se^{+6} to 3.3 %). At selenate concentrations above 2 ppm in the soil the sulphur content of the radish leaves is nearly constant. No reduction in the yield was seen with radish and rye grass as the test plants (tables 6.2 and 6.3). In the same tables will be found the absorption quotients for the added sulphate. Up to 60 % was removed by one culture of radish.

The chemical character of the sulphur in the plants was determined in aqueous extracts. In the green part of the plants practically all the sulphur was extractable with water, while 20 to 40 % remained in the extracted

Table 6.2

Plant yield, chemical character of sulphur in the plants, and absorption quotient for the added sulphate at increasing additions of selenate to the soil. The test plant was radish, and 100 ppm S as sulphate labelled with S-35 was added. Pot experiment No. 8. All figures are on a dry basis.

Se^{+6} added to the soil, ppm	Radish, leaves					Radish, roots				
	Yield, g/6 pots	Total S, %	SO_4-S, %	H_2O-sol. org. S, %	Absorption quotient	Yield, g/6 pots	Total S, %	SO_4-S, %	H_2O-sol org. S, %	Absorption quotient
0	8.6	1.21	1.15	0.087	0.17	9.5	0.42	0.24	0.074	0.067
0.2	8.6	1.23	1.16	0.089	0.18	7.9	0.44	0.23	0.073	0.058
0.5	8.5	1.26	1.18	0.086	0.18	10.0	0.42	0.20	0.058	0.069
1.0	9.4	1.63	1.55	0.077	0.26	10.5	0.46	0.24	0.070	0.081
1.5	8.8	1.90	2.16	0.099	0.41	9.8	0.49	0.38	0.105	0.080
2.0	10.2	2.98	2.52	0.073	0.51	11.5	0.53	0.33	0.070	0.102
3.0	9.6	2.94	2.86	0.080	0.47	10.0	0.56	0.35	0.062	0.093
4.0	9.4	3.06	2.96	0.068	0.48	10.5	0.56	0.34	0.064	0.098
5.0	8.9	3.16	3.33	0.065	0.47	9.5	0.64	0.41	0.090	0.101
7.5	8.3	3.18	3.12	0.055	0.44	9.8	0.54	0.33	0.067	0.088
10.0	8.5	3.26	2.85	0.056	0.46	7.4	0.60	0.40	0.069	0.074

The regression of water-soluble, organic S on ppm Se^{+6} in the soil:
$Y = (0.0760 \pm 0.0018) - (0.00380 \pm 0.00060)\ (x_i - 3.1545)$
or $Y = 0.0880 - 0.0038x_i$
$P << 0.1\%$ for b = 0 (where b is the slope)

The regression of water-soluble, organic S on ppm Se^{+6} in the soil:
b is not significantly different from zero.

Table 6.3

Plant yield, chemical character of sulphur in plants, and absorption quotient for the added sulphate at increasing additions of selenate to the soil. The test plant was rye grass, and 100 ppm S as sulphate labelled with S-35 was added. Pot experiment No. 8. All figures are on a dry basis.

Se^{+6} added to the soil, ppm	Yield, g/6 pots	Total S, %	SO_4-S, %	H_2O-sol. org. S, %	Absorption quotient
0	*22.0*	*0.61*	*0.58*	*0.019*	*0.22*
0.5	21.5	0.60	0.52	0.020	0.22
1.0	20.3	0.65	0.58	0.023	0.22
2.0	22.3	0.78	0.72	0.021	0.29
5.0	22.2	1.04	1.00	0.021	0.38

roots. At least part of this residual sulphur is not necessarily insoluble in water but may have been retained in the voluminous root pulp. Sulphate in the extract was precipitated with Ba^{++}. The sulphur remaining in solution was assumed to be organically bound, presumably as amino acids and peptides, because no part of the aqueous extract could be precipitated with trichloroacetic acid.

The largest part of the sulphur in the extracts was present as sulphate, and the amount increased with the increasing sulphur concentration (tables 6.2 and 6.3). The amount of water-soluble, organic sulphur in radish leaves decreased, however, with the increasing absorption of sulphur (table 6.2). The regression of this fraction on the selenium addition to the soil is linear and highly significant. In the roots, however, the amount of water-soluble, organic sulphur seems to be constant. As compared with the leaves, a larger part of the sulphur in the roots is contained in this fraction. In rye grass (table 6.3) no decrease in the water-soluble, organic sulphur was found within the applied selenate concentrations. The amounts were close to 0.02 % for all selenate additions.

6.2.2. Varied Selenite Addition

As has been seen previously (chapter 4), a selenite addition to the soil results in a selenium concentration in radish leaves which is about one fifth or less than that obtained with the same addition as selenate. For the root the corresponding factor is one third or less. This means that a supply of 10 ppm Se^{+6} should be compared with 30 to 50 ppm Se^{+4} in the soil. Hence selenite additions corresponding to up to 50 ppm Se were used (pot experiment No. 9). The growth at 50 ppm Se was extremely poor, already at a concentration of 5 ppm Se, the yield begins to decrease (table 6.4).

Table 6.4

Plant yield, chemical character of sulphur in plants, and absorption quotient for added sulphate at increasing additions of selenite to the soil. The test plant was radish, and 100 ppm S as sulphate labelled with S-35 was added. Pot experiment No. 9. All figures are on a dry basis.

Se^{+4} added to the soil, ppm	Radish leaves					Radish root				
	Yield, g/6 pots	Total S, %	SO_4-S, %	H_2O-sol. org. S, %	Absorption quotient	Yield, g/6 pots	Total S, %	SO_4-S, %	H_2O-sol. org. S, %	Absorption quotient
0	15.4	0.65	0.31	0.069	0.166	6.6	0.40	0.10	0.086	0.044
1	14.7	0.67	0.31	0.065	0.164	7.1	0.39	0.09	0.090	0.044
2	16.2	0.59	0.29	0.062	0.160	8.7	0.38	0.09	0.080	0.055
5	12.6	0.51	0.22	0.054	0.107	7.6	0.36	0.08	0.081	0.046
10	10.1	0.37	0.10	0.054	0.062	3.7	0.39	0.10	0.098	0.024
20	2.6	0.42	0.11	0.067	0.018	0.7	0.47	0.09	0.116	0.010
50	0					0				

Increasing additions of selenite did not result in an increase in the sulphur concentration in the plant (table 6.4) as was the case for selenate. Furthermore, when the plant yield decreased at the higher selenite levels, the sulphur content in the leaves decreased as well.

The percentage of water-soluble sulphur in the plants was generally lower than in the previous experiment with selenate, where it was constantly close to 100 %. With selenite it decreased from 60 % at no addition to 40 % at the higher selenite levels. In the roots 50 % of the sulphur was found in the extracts. The addition of increasing amounts of selenite resulted in a nearly constant level of water-soluble, organic sulphur contrary to what was found after selenate additions. Further, after selenite addition the soluble, organic fraction constituted a much larger part of the soluble sulphur than in the selenate series. In the roots the amounts of sulphate-S and soluble, organic sulphur were equal.

6.3. Discussion

Addition of selenate to the soil causes an increased absorption of sulphur, and the increase is associated with the size of the addition.

The increased amount of sulphur in the plant leaves is mainly stored as sulphate. This together with the decrease in the amount of water-soluble, organic sulphur seen in the radish leaves indicates that a blocking of the sulphate reduction occurs.

A principal difference in the reduction pathways of sulphate and selenate occurs after the formation of APS*) and APSe*), respectively. While the APS is transformed into PAPS*) the existence of the selenium analog has not been demonstrated (Wils 1958, Nis 1964). The present results may be explained by a consumption of the available ATP by the selenate through the formation of APSe + PP*). Consequently, little or no APS and PAPS can be formed, and as the sulphate reduction pathway goes on from this compound, no new organic sulphur compounds will be formed. Hence the decrease in the amount of water-soluble, organic S which is seen in table 6.1. Owing to a lack of reduced sulphur compounds the absorption of sulphate by the plant is continued. This results in the increase from 1 to 3 % of total sulphur in the leaves. Above this concentration, the plant does not seem to be able to increase the sulphur concentration further, though the selenium concentration is still increasing. In rye grass a decrease in the organic S was not ascertained, but this may be due to the smaller selenium concentration in the rye grass as compared with the radish.

Selenite additions to the soil do not result in an increase in the sulphur content of the plants nor do they seem to affect the sulphate reduction (fig.

*) APS (and APSe): adenosine 5′-phosphosulphate (selenate)
PAPS: 3′-phosphoadenosine 5′-phosphosulphate
PP: pyrophosphate

6.1 and table 6.4). On the contrary, the concentration of total sulphur and of SO_4-S in the leaves of radish decreases as soon as the yield is affected by the selenite addition. In the root a pink colour was seen at the highest selenite additions. This effect, also observed by Hurd Karrer (Hur 1937b), presumably is caused by the reduction of selenite to elemental selenium.

7. SUMMARY and CONCLUDING REMARKS

The element selenium is an essential micro-nutrient for higher animals. The present work applies highly sensitive radio-chemical and radio-analytical methods to the study of selenium in Danish soils and plants and to problems related to fertilization with selenium.

An analytical survey (chapter 3) shows that in most Danish agricultural crops the concentrations of selenium are marginal. In pasture species the concentrations vary around the minimum acceptable value, in beets, potatoes, and cereals they are usually lower than that. Only in a few cases, notably the Cruciferae family, are the concentrations above the minimum required for the production of healthy animals. Somewhat surprisingly, serious symptoms of selenium deficiency are only encountered occasionally in Denmark. The reason is probably that the ration is often supplemented with (selenium-rich) foodstuffs such as oilcakes and fishmeal. A somewhat increased selenium level in Danish foodstuffs seems desirable, and a possible solution is to add small amounts of selenium compounds to the common fertilizers.

A number of experiments (chapter 4) deal with the uptake in crops of selenium added to the soils. The resulting concentration depends mainly on the plant species and on the amount and oxidation state of the added selenium. Of little importance are the solubility of the selenium salt, the stage of development of the plant at harvest, and (as long as only mineral soils are considered) the soil type and its state of fertilization. For a given addition of selenium (as selenate or selenite) to the soil, the resulting concentration in the plants will vary by a factor of about 10 depending on the species (concentrations increase from cereals to members of the Cruciferae family), and by a factor of 2 depending upon the soil type (concentrations increase with decreasing clay content). Altogether it should for Danish mineral soils be possible from a restricted number of field trials to predict the resulting concentration in plants to within a factor of 3. Such an accuracy would suffice for practical applications.

The choice of a selenium compound suitable for field use is not an obvious one. Selenates have high utilization, and remaining selenate will probably be leached with a risk of bringing selenium into the ground water. Another solution is to use selenites, which are taken up by the plants in small amounts only, and which tend to accumulate in the soils.

A number of experiments (chapters 4 and 5) dealt with the chemical forms of selenium in soils and plants. In the soil, selenium compounds are

transformed predominantly into the +4 (selenite) oxidation state irrespective of whether selenate or selenite have been added to begin with. The oxidation states +6, 0, −2 as well as organic compounds are also found. Experiments on the chemical forms of selenium in plants were carried out with radish as the test plant. The selenite fraction appears to be larger than indicated in older works. Of theoretical, rather than of practical, interest is an increase in sulphur absorption found after the application of large amounts of selenate (chapter 6). The additional sulphur turns out to be present as sulphate, and therefore a likely explanation of the effect is that the selenate ion blocks the pathway for the sulphur reduction.

From a phenomenological point of view, answers are now at hand to the main problems in connection with selenium and soils. Further laboratory experiments will serve mainly to fill in details, although one may still hope for a new solution to the challenging problem of the ideal compound for selenium fertilization. The logical next step would now be to undertake field experiments on a larger scale. The sensitive analytical techniques available (reactor-neutron activation, fluorometry) will make it possible to keep such a "pilot" experiment under complete control.

APPENDIX

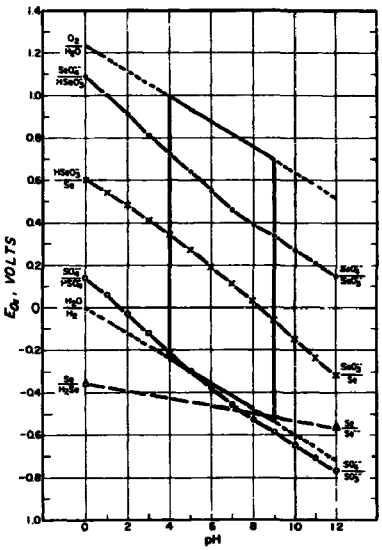

Fig. A.1. Equilibria between the different oxidation states of selenium and sulphur as a function of pH. The potentials are calculated for total concentrations equal to 10^{-3} M. The curves O_2/H_2O and H_2O/H_2 indicate the highest and lowest redox potentials normally obtainable in soils. Thus the parallelogram gives the area within which most soils will be found. It is seen that contrary to sulphur (which will normally be present as sulphate) selenium may be present also in the oxidation states +4 and 0 and even in -2.

Table A.1

Solubilities of some selenium and sulphur compounds (Gme, Land 1962, Hod 1962)

Selenium compound	Solubility*) g/100 ml H_2O	Sulphur compound	Solubility*) g/100 ml H_2O
Na_2SeO_4	84^{35} 72.8^{100}	Na_2SO_4	4.8^{0} 42.7^{100}
K_2SeO_4	110.5^{0}	K_2SO_4	12^{25}
$CaSeO_4$	7.9^{6} 5.4^{67}	$CaSO_4$	0.209^{3}
$SrSeO_4$	0.136^{10}	$SrSO_4$	0.0114^{30}
$BaSeO_4$	0.0118	$BaSO_4$	0.00022^{18}
$CuSeO_4 \cdot 5H_2O$	25.7^{15}	$CuSO_4 \cdot 5H_2O$	31.6^{0} 203^{100}
$Na_2SeO_3 \cdot 5H_2O$	s.	Na_2SO_3	12.5^{0}
K_2SeO_3	58.0	$K_2SO_3 \cdot 2H_2O$	100
$CaSeO_3$		$CaSO_3 \cdot 2\,H_2O$	0.0043^{18}
$SrSeO_3$		$SrSO_3$	0.0033^{17}
$BaSeO_3$	0.018^{10}	$BaSO_3$	0.02
$CuSeO_3 \cdot 2H_2O$	i.		
$FeSeO_3$		$FeSO_3 \cdot 3H_2O$	v.sl.s.

*) The solubilities are stated for 20°C, unless otherwise indicated by superscript.

ACKNOWLEDGEMENTS

This work has been carried out at the Agricultural Research Department at Risø. I am grateful for the excellent working conditions provided and especially indebted to the Department Head, Jens Sandfær, dr. agro., for his continual support and interest in this work. I have also benefited from discussions with many colleagues in the Department, especially Arna Andersen, dr. agro., and, in recent years, Gunnar Gissel Nielsen, lic. agro..

The experiments described here have to a large extent been dependent on representative samples of plant material. For providing such samples I am indebted to the Experimental Stations Abed, Askov, Aarslev, Blangstedgaard, Borris, Centralgaarden, Højer, St. Jyndevad, Kannikegaarden, Lyngby, Lundgaard, Rønhave, Studsgaard, Tylstrup and Ødum.

Finally, during the preparation of this report, I have been able to take counsel with Professors F. Steenbjerg, dr. agro., and K. Rasmussen, dr. agro.. I wish to thank them for their advice and help.

REFERENCES

Abu-Erreisch, G.M., Whitehead, E. I. and Olson, O. E.: Evolution of volatile selenium from soils. Soil Sci. **106** 415-20 (1968).

Allaway, W. H. and Hodgson, J. F.: Selenium in forages as related to the geographic distribution of muscular dystrophy in livestock. J. Anim. Sci. **11** 609-20 (1964a).

Allaway, W. H. and Cary, Earle E.: Determination of submicrogram amounts of selenium in biological materials. Anal. Chem. **36** 1359-62 (1964b).

Allaway, W. H., Moore, D. P., Oldfield, J. E., and Muth, O. H.: Movement of physiological levels of selenium from soils through plants to animals. J. Nutr. **88** 411-18 (1966).

Andrews, E. D., Grant, A. B. and Stephenson, B. J.: Weight response of sheep to cobalt and selenium in relation to vitamin B_{12} and selenium concentrations in liver and kidney. N. Z. J. Agr. Res. **7** 17-27 (1964).

Arnon, D. I. and Stout, P. R.: The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol. **14** 371-75 (1939).

Asher, C. J., Evans, C. S. and Johnson, C. M.: Collection and partial characterization of volatile selenium compounds from Medicago saliva L., Austr. J. Biol. Sci. **20** 737-48 (1967).

Bardsley, C. E. and Lancaster, J. D.: Sulfur. Methods of soil analysis. Edited by C. A. Black (American Society of Agronomy Inc., Publ., Madison, Wis., 1965) 1102-16.

Beath, O. A., Draize, J. H., Eppson, H. F., Gilbert, C. S., and McCreary, O. C.: Certain poisonous plants of Wyoming activated by selenium and their associates with respect to soil types. J. Amer. Pharm. Ass. (Sci. Ed.) **23** 94-97 (1934).

Beath, O. A., Eppson, H. F. and Gilbert, C. S.: Selenium distribution in and seasonal variation of type of vegetation occurring on seleniferous soils. J. Amer. Pharm. Ass. **26** 394-405 (1937).

Beath, O. A. and Eppson, H. F.: The form of selenium in some vegetation. Wyom. Agr. Exp. Sta. Bull. **278** 1-15 (1947).

Beeson, K. C.: Occurrence and significance of selenium in plants. In: M. S. Anderson et al. "Selenium in Agriculture". U. S. Dep. Agr., Agr. Handb. **200** 34-40 (1961).

Behrens, H.: Lehrbuch der Schafkrankheiten. (Verlag Parey, Berlin, 1962) 200-202.

Bieri, J. G., Dam, H., Prange, Inger, and Søndergaard, E.: Effect of dietary selenium dioxide, cystine, ethoxyquin and vitamin E on lipid autoxidation in chick tissues. Acta Physiol. Scand. 52 36-43 (1961).

Bisbjerg, B. and Gissel-Nielsen, G.: The uptake of applied selenium by agricultural plants. I. The influence of soil type and plant species. Plant Soil 31 287-98 (1969).

Bisbjerg, B. Jochumsen, P. and Rasbeck, N. O.: Selenium content in organs, milk and fodder. Nord. Vet.-Med. 22 532-35 (1970).

Black, C. A.: Soil - Plant relationships. 2nd edition. (Wiley, New York, 1968) 792 pp.

Blanchar, R. W., Rehm, G. and Caldwell, A. C.: Sulfur in plant materials by digestion with nitric and perchloric acid. Soil Sci. Soc. Amer. Proc. 29 71-72 (1965).

Bock, R. and Jacob, D.: Die Bestimmung von Selenspuren. Z. Anal. Chem. 200 81-134 (1964).

Bowen, H. J. M. and Cawse, P. A.: The determination of selenium in biological material by radioactivation. Analyst 88 721-26 (1963a).

Bowen, H. J. M. and Gibbons, D.: Radioactivation analysis (Oxford, Clarendon Press, 1963b) 295 pp.

Bowen, H. J. M.: Trace elements in biochemistry (Academic Press, London, 1966) 241 pp.

Brown, M. J. and Carter, D. L.: Leaching of added selenium from alkaline soils as influenced by sulfate. Soil Sci. Soc. Amer. Proc. 33 563-65 (1969).

Broyer, T. C., Lee, D. C. and Asher, C. J.: Selenium nutrition of green plants. Effect of selenite supply on growth and selenium content of alfalfa and subterranean clover. Plant Physiol. 41 1425-28 (1966).

Brunfelt, A. O. and Steinness, E.: Determination of selenium in standard rocks by neutron activation analysis. Geochim. Cosmochim. Acta 31 283-85 (1967).

Buketov, E. A., Moiseevich, O. Yu. and Ugoreta, M. Z.: Separation and determination of quadri- and sexavalent selenium. (Zavod. Lab. 30 787-88 (1964)) Anal. Abstr. 12 5756 (1965).

Butler, G. W. and Peterson, P. J.: Aspects of the faecal excretion of selenium by sheep. N. Z. J. Agr. Res. 4 484-91 (1961).

Byers, H. G.: Selenium occurrence in certain soils in the United States, with a discussion of related topics. I. U.S. Dep. Agr. Tech. Bull. 482 1-47 (1935).

Byers, H. G., Miller, J. T., Williams, K. T., and Lakin, H. W.: Selenium occurrence in certain soils of the United States, with a discussion of related topics. III. U.S. Dep. Agr. Tech. Bull. 601 1-75 (1938).

Carter, D. L., Brown, M. J., Allaway, W. H., and Cary, Earle E.: Selenium content of forage and hay crops in the Pacific Northwest. Agron. J. 60 532-34 (1968).

Carter D. L., Brown, M. J. and Robbins, C. W.: Selenium concentrations in alfalfa from several sources applied to a low selenium, alkaline soil. Soil Sci. Soc. Amer. Proc. 33 715-18 (1969).

Carter, D. L., Robbins, C. W. and Brown, M. J.: Selenium concentrations in forage on some high northwestern ranges. J. Range Manage. 23 234-38 (1970).

Cary, Earle E., Wieczorek, G. A. and Allaway, W. H.: Reactions of selenite-selenium added to soils that produce low-selenium forages. Soil Sci. Soc. Amer. Proc. 31 21-26 (1967).

Cary, Earle E. and Allaway, W. H.: The stability of different forms of selenium applied to low-selenium soils. Soil Sci. Soc. Amer. Proc. 33 571-74 (1969).

Cousins F. B.: A fluorimetric microdetermination of selenium in biological material. Aust. J. Exp. Biol. Med. Sci. 38 11-16 (1960).

Cousins, F. B. and Cairney, I. M.: Some aspects of selenium metabolism in sheep. Aust. J. Agr. Res. 12 927-43 (1961).

Crouthamel, C. E. (editor): Applied gamma-ray spectrometry. (Oxford, Pergamon, 1960) 443 pp.

Cukor, P., Walzcyk, J. and Lott, P. F.: The application of isotopic dilution analysis to the fluorimetric determination of selenium in plant material. Anal. Chim. Acta 30 473-82 (1964).

Cummins, L. M., Martin, J. L., Maag, Grace W., and Maag, D. D.: A rapid method for the determination of selenium in biological material. Anal. Chem. 36 382-84 (1964).

Dahl, J. B. and Steinness, E.: Determination of selenium in animal tissue by neutron activation analysis. KR-95 (Kjeller Report) 1965, 6 pp.

Dam, H., Kofoed Nielsen, Gunhild, Prange, Inge, and Søndergaard, E.: The exudative diathesis produced by Torula yeast. Experientia 13 493-94 (1957a).

Dam, H. and Søndergaard, E.: Prophylactic effect of selenium dioxide against degeneration (white striation) of muscles in chicks. Experientia 13 494 (1957b).

Davies, E. B. and Watkinson, J. H.: Uptake of native and applied selenium by pasture species. I. Uptake of Se by browntop, ryegrass, cocksfoot, and white clover from Atiamuri sand. N. Z. J. Agr. Res. 9 317-27 (1966a).

Davies, E. B. and Watkinson, J. H.: Uptake of native and applied selenium by pasture species. II. Effects of sulphate and of soil type on uptake by clover. N. Z. J. Agr. Res. 9 641-51 (1966b).

Dickson, R. C. and Tappel, A. L.: Effects of selenocystine and selenomethionine on activation of sulfhydryl enzymes. Arch. Biochem. Biophys. 131 100-110 (1969).

Duftschmid, K. E. and Leibetseder, J.: Investigations of the connection between vitamin E deficiency and the selenium content in animal organs. Nuclear Activation Techniques in the Life Sciences, Amsterdam, 8-12 May 1967. (IAEA, Vienna, 1967) 353-63.

Dye, W. B., Bretthauer, E. and Blincoe, C.: Fluorometric determination of selenium in plants and animals with 3,3'-diaminobenzidine. Anal. Chem. 35 1687-93 (1963).

Ehlig, C. F., Allaway, W. H., Cary, Earle E., and Kubota, J.: Differences among plant species in selenium accumulation from soils low in available selenium. Agron. J. 60 43-47 (1968).

Evans, Christine S., Asher, C. J. and Johnson, C. M.: Isolation of dimethyl diselenide and other volatile selenium compounds from Astragalus racemosus (Pursh.). Aust. J. Biol. Sci. 21 13-20 (1968).

Ewan, R. C., Baumann, C. A. and Pope, A. L.: Determination of selenium in biological materials. J. Agr. Food Chem. 16 212-15 (1968).

Fineman, I., Ljunggren, K., Forsberg, H. G., and Erwall, L.-G.: Activation analysis for selenium in ore concentrates, slags and waste gases obtained in a metallurgical industry. Int. J. Appl. Radiat. Isotop. 5 280-88 (1959).

Fink, H., Schlie, Ilse and Schwarz, K.: Über die ernährungsbedingte Lebernekrose und den Selengehalt von Milchpulvern verschiedener Herkunft. Z. Naturforsch. 22b 1144-49 (1967).

Fleming, G. A. and Walsh, T. : Selenium occurrence in certain Irish soils and its toxic effects on animals. Proc. Royal Irish Acad., Sect. B 58 151-66 (1957).

Fleming, G. A.: Selenium in Irish soils and plants. Soil Sci. 94 28-35 (1962a).

Fleming, G. A.: Some factors affecting the uptake of selenium by plants. Irish J. Agr. Res. 1 131-38 (1962b).

Fleming, G. A.: Personal communication 1966.

Fogg, D. N. and Wilkinson, N. T.: The determination of selenium in effluents. Analyst 81 525-31 (1956).

Franke, K. W. and Moxon, A. L.: The toxicity of orally ingested arsenic, selenium, tellurium, vanadium and molybdenum. J. Pharmacol. Exp. Ther. 61 89-102 (1937a).

Franke, K. W. and Painter, E. P.: Effect of sulfur additions on seleniferous soils. Binding of selenium by soil. Ind. Eng. Chem. 29 591-95 (1937b).

Franke, K. W. and Painter, E. P.: A study of the toxicity and selenium content of seleniferous diets: With statistical consideration. Cereal Chem. 15 1-24 (1938).

Friedlander, G. and Kennedy, J. W.: Nuclear and radiochemistry. (Wiley, *New York, 1955).*

Frost, D. V.: Significance of the symposium: International Symposium on Selenium in Biomedicine, Oregon State University, 1966. Edited by O. H. Muth (AVI Publ. Co., Westport, Conn., 1967) 22.

Ganje, T. J. and Whitehead, E. J.: Evolution of volatile selenium from Pierre Shale supplied with selenium-75 as selenite or selenate. Proc. S. Dakota Acad. Sci. 37 81-84 (1958a).

Ganje, T. J. and Whitehead, E. I.: Selenium uptake by plants as affected by the forms of selenium in the soil. Proc. S. Dakota. Acad. Sci. 37 85-88 (1958b).

Ganther, H. E.: The fate of selenium in animals. World Rev. of Nutr. and Diet. 5 338-66 (1965).

Gardiner, M. R., Armstrong, J., Fels, H., and Glencross, R. N.: A preliminary report on selenium and animal health in Western Australia. Aust. J. Exp. Agr. Anim. Husb. 2 261-69 (1962).

Gardiner, M. R. and Gorman, R. C.: Further observations on plant selenium levels in Western Australia. Aust. J. Exp. Agr. Anim. Husb. 3 284-89 (1963).

Geering, H. G., Cary, E. E., Jones, L. H. P., and Allaway, W. H.: Solubility and redox criteria for the possible forms of selenium in soils. Soil Sci. Soc. Amer. Proc. 32 35-40 (1968).

Gibbons, D. and Simpson, H.: The use of short-lived radioactive isotopes in an activation analysis service programme. Production and Use of Short-lived Radioisotopes from Reactors, Proceedings Vienna 5-9 November, 1962 (IAEA, Vienna, 1963) 2 95-109.

Gissel-Nielsen, G.: Personal communication, 1968.

Gissel-Nielsen, G.: Selenindholdet i foderafgroder. Ugeskrift for agronomer 114 962-64 (1969).

Gissel-Nielsen, G.: Loss of selenium in drying and storage of agronomic plant species. Plant Soil 32 242-45 (1970a).

Gissel-Nielsen, G. and Bisbjerg, B.: The uptake of applied selenium by agricultural plants. II. The utilization of various selenium compounds. Plant Soil 32 382-96 (1970b).

Gissel-Nielsen, G.: Selenium content of some fertilizers and their influence on the uptake of selenium in plants. J. Agr. Food Chem. 19 564-66 (1971).

Gissel-Nielsen, G.: Influence of pH and texture of the soil on plant uptake of added selenium. J. Agr. Food Chem. 19 1165-67 (1971a).

Gmelins Handbuch der Anorganischen Chemie, 8th ed. (Verlag Chemie, Weinheim/Bergstrasse).

Goldschmidt, V. M.: Geochemistry (Oxford University Press, London, 1954) 730 pp.

Gorsuch, T. T.: Radiochemical investigations on the recovery for analysis of trace elements in organic and bioligical materials. Analyst **84** 135-73 (1959).

Grant, A. B.: Determination of selenium in biological material. N. Z. J. Sci. **6** 577-88 (1963).

Grant, A. B.: Pasture top-dressing with selenium. N. Z. J. Agr. Res. **8** 681-90 (1965).

Grant, C. A. and Thafvelin, B.: Selen och hepatosis diaetetica hos svin. Nord. Veterinärmed. **10** 657-63 (1958).

Gregers-Hansen (Bisbjerg), B.: Application of radioactivation analysis for the determination of selenium and cobalt in soils and plants. 8th International Congress of Soil Science. Transactions, Bucharest 31 August - 9 September, 1964. (Publishing House of the Academy of the Socialist Republic of Romania, Bucharest, 1964). **3** 63-70.

Guinn, V. P.: The determination of traces of Se, V, Sr, and As in biological samples by neutron-activation analysis (with special reference to the instrumental detection of 17-second Se 77m). In: L'Analyse par radioactivation et ses applications aux sciences biologiques. Médecine, agronomie, biochimie. Saclay, Sept. 1963. (Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette, 1964), 69-82.

Haahr, V. and Ølgaard, P. L.: Comparative experimental and theoretical investigations of the neutronic method for measuring the water content in soil. In: Isotopes and radiation in soil-plant nutrition studies. Ankara, 28 June - 2 July 1965 (IAEA, Vienna, 1965) 129-146.

Hamilton, J. W. and Beath, O. A.: Selenium uptake and conversion by certain crop plants. Agron. J. **55** 528-31 (1963a).

Hamilton, J. W. and Beath, O. A.: Uptake of available selenium by certain range plants. J. Range Manage. **16** 261-64 (1963b).

Hamilton, J. W. and Beath, O. A.: Amount and chemical form of selenium in vegetable plants. J. Agr. Food Chem. **12** 371-74 (1964).

Hartley, W. J. and Grant, A. B.: A review of selenium responsive diseases of New Zealand livestock. Fed. Proc. **20** 679-88 (1961).

Hartley, W. J.: Levels of selenium in animal tissues and methods of selenium administration. 1st International Symposium on Selenium in Biomedicine, Oregon State University, 1966. Edited by O. H. Muth. (AVI Publ. Co., Westport, Conn. 1967) 79-96.

Hashimoto, Y. and Winchester, J. W.: Selenium in the atmosphere. Environ. Sci. Technol. **1** 338-40 (1967).

Henriksen, Aa. and Jensen, J.: Sammenligning af metoder til bestemmelse af pH samt fosfor og kalium i jord. Tidskr. Planteavl **73** 331-45 (1969).

Hodgman, C. D., Weast, R. C., Shankland, R. S., and Selby, S. M.: Handbook of Chemistry and Physics (The Chem. Rubber Publ. Co., Cleveland, Ohio, 1962).

Hoffmann, I., Westerby, R. J. and Hidiroglou, M.: Precise fluorometric microdetermination of selenium in agricultural materials. J. Ass. Offic. Anal. Chem. 51 1039-42 (1968).

Hopkins, L. L. jr. and Majaj, A. S.: Selenium in human nutrition. 1st International Symposium on Selenium in Biomedicine, Oregon State University, 1966. Edited by O. H. Muth (AVI Publ. Co., Westport, Conn., 1967) 203-13.

Horn, M. J. and Jones, D. B.: Isolation from Astragalus pectinatus of a crystalline amino acid complex containing selenium and sulfur. J. Biol. Chem. 139 649-60 (1941).

Horwitt, M. K.: Role of vitamin E, selenium, and polyunsaturated fatty acids in clinical and experimental muscle disease. Fed. Proc. 24 68-72 (1965).

Hurd-Karrer, Annie M.: Inhibition of selenium injury to wheat plants by sulfur. Science 78 560 (1933).

Hurd-Karrer, Annie M.: Selenium injury to wheat plants and its inhibition by sulfur. J. Agr. Res. 49 343-57 (1934).

Hurd-Karrer, Annie M.: Selenium absorption by crop plants as related to their sulphur requirement. J. Agr. Res. 54 601-08 (1937a).

Hurd-Karrer, Annie M.: Comparative toxicity of selenates and selenites to wheat. Am. J. Bot. 24 720-28 (1937b).

Hurd-Karrer, Annie M.: Relation of sulphate to selenium absorption by plants. Am. J. Bot. 25 666-75 (1938).

Iwantscheff, G.: Das Dithizon und seine Anwendung in der Mikro- und Spurenanalyse. (Verlag Chemie, Weinheim/Bergstr., 1958) 272 pp.

Jenkins, K. J. and Hidiroglou, M.: The incorporation of Se^{75}-selenite into dystrophogenic pasture grass. The chemical nature of the seleno compounds formed and their availability to young ovine. Can. J. Biochem 45 1027-40 (1967).

Johnson, C. M., Asher, C. J. and Broyer, T. C.: Distribution of selenium in plants. 1st International Symposium on Selenium in Biomedicine, Oregon State University, 1966. Edited by O. H. Muth (AVI Publ. Co., Westport, Conn., 1967) 57-75.

Johnson, R. R. and Whitehead, E. I.: Growth and selenium content of wheat plants as related to the selenite selenium content of soil. Proc. S. Dakota Acad. Sci. 30 130-36 (1951).

Jones, G. B. and Belling, G. B.: The movement of copper, molybdenum, and selenium in soils as indicated by radioactive isotopes. Aust. J. Agr. Res. 18 733-40 (1967).

Kelleher, W. J. and Johnson, M. J.: Determination of traces of selenium in organic matter. Combined spectrophotometric and isotope dilution method. Anal. Chem. 33 1429-32 (1961).

Kiermeier, F. and Wigand, W.: Bestimmung von Selen in Milch. Z. Lebensm.-Unter.-Forsch. 136 158-66 (1968).

Koch, R. C.: Activation analysis handbook (Academic Press, New York 1960) 219 pp.

Koch, R. C. and Roesmer, J.: Application of activation analysis to the determination of trace-element concentrations in meat. J. Food Sci. 27 309-20 (1962).

Kofoed, A. Dam and Lindhard, J.: Mineralstofbortførsel fra græsdækket jord i lysimetre. Tidsskr. Planteavl 72 417-37 (1968).

Krauskopf, K. B.: Sedimentary deposits of rare metals. Econ. Geol. 50th Anniversary Vol. 411-63 (1956).

Krober, O. A. and Howell, R. W.: Determination of sulfur in plant materials. J. Agr. Food Chem. 6 591-92 (1958).

Kubota, J., Allaway, W. H., Carter, D. L., Cary, Earle E., and Lazar, V. A.: Selenium in crops in the United States in relation to selenium-responsive diseases of animals. J. Agr. Food Chem. 15 448-53 (1967).

Kuroda, R.: Activation analysis of selenium using the 3.9 min Se-79m isomer. TID 17272, 68-69 (1962).

Kylin, A.: The uptake and metabolism of sulphate by deseeded wheat plants. Physiol. Plant. 6 775-95 (1953).

Kylin, A.: The incorporation of radio-sulphur from external sulphate into different sulphur fractions of isolated leaves. Physiol. Plant. 13 366-79 (1960).

Lakin, H. W.: Selenium content of soils. In: M. S. Anderson et al. "Selenium in agriculture". U.S. Dep. Agr., Agr. Hand. 200 27-34 (1961).

Lakin, H. W. and Davidson, D. F.: The relation of the geochemistry of selenium to its occurrence in soils. 1st International Symposium on Selenium in Biomedicine, Oregon State University, 1966. Edited by O. H. Muth (AVI Publ. Co., Westport, Conn., 1967) 27-56.

Lamm, C. G.: Mangans kemi i jordbunden (Akademisk forlag, Copenhagen, 1964) 248 pp.

Landolt-Börnstein: Zahlenwerte und Funktionen. 6th ed. Vol. II 2 (Springer Verlag, Berlin, 1962).

Lane, J. C. and Fleming, G. A.: The effect of stage of maturity and season on the uptake of selenium, molybdenum and copper by perennial rye grass. Trans. Second Fourth Comm. Int. Soc. Soil Sci., Aberdeen, 289-97 (1966a),

Lane, J. C.: Determination of selenium in soil and biological materials. Irish J. Agr. Res. 5 177-83 (1966b).

Lannek, N., Lindberg, P., Nilsson, G., Nordström, G., Orstadius, Kerstin, and Åberg, B.: Production of muscular dystrophy in pigs by feeding certain grains. Zentralbl. Veterinärmed. 7 403-10 (1960).

Leddicotte, G. W.: The radiochemistry of selenium (National Academy of Science, Nuclear Science Series, USAEC) NAS–NS 3030 (1961) 49 pp.

Lederer, C. M., Hollander, J. M. and Perlman, I.: Table of isotopes. 6th edition (Wiley, New York, 1967) 594 pp.

Leggett J. E. and Epstein, E.: Kinetics of sulfate absorption by barley roots. Plant Physiol. 31 222-26 (1956).

Lessard, J. R., Hidiroglou, M., Carson, R. B., and Dermine, P.: Intra-seasonal variations in the selenium content of various forage crops at Kapuskasing, Ontario. Can. J. Plant Sci. 48 581-85 (1968).

Levesque, M. and Vendette, E. D.: Selenium determination in soil and plant materials. Can. J. Soil Sci. 51 85-93 (1971).

Lewis, B. G., Johnson, C. M. and Delwiche, C. C.: Release of volatile selenium compounds by plants. Collection procedures and preliminary observations. J. Agr. Food Chem. 14 638-40 (1966).

Lindberg, P. and Siren, M.: Fluorometric selenium determinations in the liver of normal pigs and in pigs affected with nutritional muscular dystrophy and liver dystrophy. Acta Vet. Scand. 6 59-64 (1965).

Lindberg, P. and Tanhuanpää, E.: Nutrionelt betingad muskeldegeneration hos svin. Kongressberättelse. 10. Nordiska Veterinärmötet, Stockholm, 15-18 August 1966. (Sveriges Veterinärförbund, Stockholm 1966) 832-38.

Lindberg, P.: Selenium determination in plant and animal material, and in water. Acta Vet. Scand., Suppl. 23 (1968) 48 pp.

Lindberg, P. and Bingefors, S.: Selenium levels of forages and soils in different regions of Sweden. Acta Agr. Scand. 20 133-36 (1970).

Lippert, J.: Low level counting. Risö Report No. 44 (1963) 72 pp.

Ludvigsen, J. B.: Problemer i svineproduktionen. Medlemsblad Danske Dyrlægeforening 47 87-98 (1964).

Ludvigsen, J. B.: "Muskeldegeneration" hos svin. Nord. Jordbrugsforsk. 47 318-22 (1965).

Lunde, G.: Analysis of arsenic and selenium in marine raw materials. J. Sci. Food Agr. 21 242-47 (1970).

Løn, O.: Muskeldegeneration hos lam. Medlemsblad Danske Dyrlægeforening 48 108-11 (1965).

Marchés, J., Bogstra, J. F., Harmsen, A., and Boon, J. W.: Redoxpotentiaal en Bodemagressiviteit. (Corrosie-Instituut T. N. O., Circulaire 3, Dec. 1952).

Maxia, Vera and Rollier, M. A.: Determination of selenium in amino acids at the 0.1 ppm level by pulsed-neutron activation. Nucl. Appl. 3 187-90 (1967).

McConnell, K. P.: Passage of selenium through the mammary glands of the white rat and the distribution of selenium in the milk proteins after subcutaneous injection of sodium selenate. J. Biol. Chem. 173 653-57 (1948).

McConnell, K. P. and Portman, O. W.: Excretion of dimethyl selenide by the rat. J. Biol. Chem. 195 277-82 (1952).

McConnell, K. P.: Use of activation analysis in studies of selenium in the mammalian organism. Proceedings 1961 International Conference. Modern Trends in Activation Analysis, College Station, Texas, 15-16 December 1961. Edited by Mildred W. Brown. (A & M College of Texas, College Station, Texas, 1962) 137-40.

McConnell, K. P.: Metabolism of selenium in the mammalian organism. J. Agr. Food Chem. 11 385-88 (1963).

McConnell, K. P. and Roth, Dorothy M.: Passage of selenium across the placenta and also into the milk of the dog. J. Nutr. 84 340-44 (1964).

McKenzie, L. J., Whiteside, E. P. and Erickson, A. E.: Oxidation-reduction studies on the mechanism of B horizon formation in podzols. Am. Soil Sci. Soc. Proc. 24 300-305 (1960).

Miller, W. T. and Williams, K. T.: Minimum lethal dose of selenium, as sodium selenite, for horses, mules, cattle, and swine. J. Agr. Res. 60 163-73 (1940).

Molloy, J. P.: Determination of selenium in soils by a modified fluorometric method. Irish J. Agr. Res. 6 133-35 (1967).

Morette, A. and Divin, J. P.: Sur le sélénium des eaux. Analyse. Résultats. Ann. Pharm. Fr. 23 169-78 (1965).

Moxon, A. L. and Rhian, M.: Loss of selenium by various grains during storage. Proc. S. Dakota Acad. Sci. 18 20-22 (1938).

Moxon, A. L. and Rhian, M.: Selenium poisoning. Physiol. Rev. 23 305-37 (1943).

Moxon, A. L., Olson, O. E. and Searight, W. V.: Selenium in rocks, soils and plants. S. Dakota Agr. Exp. Sta. Tech. Bull. No. 2, 1950. 94 pp. (Revised May 1950).

Muth, O. H., Oldfield, J. E. and Weswig, P. H. (editors): 1st International Symposium on Selenium in Biomedicine, Oregon State University 1966 (AVI Publ. Co., Westport, Conn., 1967) 445 pp.

Nicholas, D. J. D.: Minor mineral nutrients. Ann. Rev. Plant Physiol. 12 63-90 (1961).

Nigam, S. N. and McConnell, W. B.: Seleno amino compounds from Astragalus bisulcatus. Isolation and identification of γ-L-glutamyl-Se-methylseleno-L-cysteine and Se-methylseleno-L-cysteine. Biochim. Biophys. Acta 192 185-90 (1969).

Nissim, N. E.: Determination of availability of soil selenium to plants. Bull. Res. Counc. Isr., Sect. G. 8 155 (1959).

Nissen, P. and Benson, A. A.: Absence of selenate esters and "selenolipid" in plants. Biochem. Biophys. Acta. 82 400-02 (1964).

Oelschläger, W. and Menke, K. H.: Über Selengehalte pflanzlicher, tierischer und anderer Stoffe. 1. Mitteilung: Selengehalt in Futtermitteln. Z. Ernähr.-wiss. 9 208-15 (1968).

Okada, M.: Non-destructive analysis of selenium by neutron activation followed by gamma-ray spectrometry. Nature 187 594-95 (1960).

Oksanen, H. E.: Studies on nutritional muscular degeneration (NMD) in ruminants. Acta Vet. Scand. 6 suppl. 2 (1965) 110 pp.

Olcott, H. S., Brown, W. D. and Van der Veen, J.: Selenomethionine as an antioxidant. Nature 191 1201-1202 (1961).

Oldfield, J. E., Schubert, J. R. and Muth, O. H.: Implications of selenium in large animal nutrition. J. Agr. Food Chem. 11 388-90 (1963).

Olson, O. E. and Moxon, A. L.: The availability to crop plants of different forms of selenium in the soil. Soil Sci. 47 305-11 (1939).

Olson, O. E., Whitehead, E. I. and Moxon, A. L.: Occurrence of soluble selenium in soil and its availability to plants. Soil Sci. 54 47-53 (1942).

Orstadius, Kerstin: Toxicity of a single subcutaneous dose of sodium selenite in pigs. Nature 188 1117 (1960).

Orstadius, Kerstin: Nutritional muscular dystrophy in pigs. Studies on the aetiology, diagnosis and therapy. Veterinärhögskolan, Uppsala, 1961, 28 pp.

Ostadius, Kerstin, Nordström, G. and Lannek, N.: Combined therapy with vitamin E and selenite in experimental nutritional muscular dystrophy of pigs. Cornell Vet. 53 60-70 (1963).

Parker, C. A. and Harvey, L. G.: Luminescence of some piazselenols. A new fluorimetric reagent for selenium. Analyst 87 558-65 (1962).

Peterson, P. J. and Butler, G. W.: The uptake and assimilation of selenite by higher plants. Aust. J. Biol. Sci. 15 126-46 (1962).

Peterson, P. J. and Butler, G. W.: Colloidal selenium availability to three pasture species in pot culture. Nature 212 961-62 (1966).

Peterson, P. J. and Butler, G. W.: Significance of selenocystathionine in an Australian selenium-accumulating plant, Neptunia amplexicaulis. Nature 213 599-600 (1967).

Picou, D. and Waterlow, J. C.: Estimation of small amounts of sulphate by precipitation with radioactive barium. Nature 197 1103-1104 (1963).

Plotnikov, V. I.: Coprecipitation of selenium and tellurium with metal hydroxides. Russ. J. Inorg. Chem. 5 351-54 (1960).

Rader, L. F. and Hill, W. L.: Occurrence of selenium in natural phosphates, superphosphates, and phosphoric acid. J. Agr. Res. 51 1071-83 (1935).

Rankama, K. and Sahama, T. G.: Geochemistry (University of Chicago Press, Chicago, 1950) 911 pp.

Rasbech, N. O.: Personal communication, 1965.

Rasbech, N. O., Jochumsen, P. and Veirup, N. H.: Undersøgelser over sygdomsforekomst og bedriftsforhold. Kgl. Vet.- og Landbohøjskole, Institut for Sterilitetsforskning, Årsberetning, 1968, 113-166.

Rasmussen, K.: Uorganiske svovlforbindelsers omsætninger i jordbunden. (DSR Forlag, Den kgl. Veterinær- og Landbohøjskole, København 1961), 176 pp.

Ravikovitch, S. and Margolin, M.: Selenium in soils and plants. Isr. Agr. Res. Sta., Rehovot Series 145-E 7 41-52 (1957).

Ravikovitch, S. and Margolin, M.: The effect of barium chloride and calcium sulphate in hindering selenium absorption by lucerne. Emp. J. Exp. Agr. 27 (107) 235-40 (1959).

Reid, B. L., Rahman, M. M., Creech, B. G. and Couch, J. R.: Selenium and development of exudative diathesis in chicks. Proc. Soc. Exp. Biol. Med. 97 590-93 (1958).

Robbins, C. W. and Carter, D. L.: Selenium concentrations in phosphorous fertilizer materials and associated uptake by plants. Soil Sci. Soc. Amer. Proc. 34 506-509 (1970).

Robinson, W. O.: Determination of selenium in wheat and soils. J. Ass. Offic. Agr. Chem. 16 423-24 (1933).

Rosenfeld, Irene and Beath, O. A.: Selenium. Geobotany, biochemistry, toxicity and nutrition. (Academic Press, New York, 1964) 411 pp.

Samsahl, K.: Radiochemical method for determination of arsenic, bromine, mercury, antimony, and selenium in neutron-irradiated biological material. Anal. Chem. 39 1480-83 (1967).

Schutz, D. F. and Turekian, K. K.: The investigation of the geographical and vertical distribution of several trace elements in sea water using neutron activation analysis. Geochim. Cosmochim. Acta 29 259-313 (1965).

Schwarz, K.: Production of dietary necrotic liver degeneration using American Torula yeast. Proc. Soc. Exp. Biol. Med. 77 818-23 (1951a).

Schwarz, K.: A hitherto unrecognized factor against dietary necrotic liver degeneration in American yeast (Factor 3). Proc. Soc. Exp. Biol. Med. 78 852-56 (1951b).

Schwarz, K. and Foltz, C. M.: Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79 3292-93 (1957).

Schwarz, K. and Foltz, C. M.: Factor 3 activity of selenium compounds. J. Biol. Chem. 233 245-51 (1958).

Schwarz, K., Stesney, Jo Ann and Foltz, C. M.: Relation between selenium traces in L-cystine and protection against dietary liver necrosis. Metab. Clin. Exp. 8 88-90 (1959).

Schwarz, K.: Development and status of experimental work on Factor 3-selenium. In: Nutritional significance of selenium (Factor 3). Fed. Proc. **20** 666-73 (1961).

Schwarz, K.: Role of vitamin E, selenium, and related factors in experimental nutritional liver disease. Fed. Proc. **24** 58-67 (1965).

Shrift, A. and Virupaksha, T. K.: Seleno-amino acids in selenium-accumulating plants. Biochim. Biophys. Acta **100** 65-75 (1965).

Shrift, A.: Microbial research with selenium. 1st International Symposium on Selenium in Biomedicine, Oregon State University, 1966. (Edited by O. H. Muth.) (AVI Publ. Co., Westport, Conn., 1967) 241-71.

Shrift A.: Aspects of selenium metabolism in higher plants. Ann. Rev. Plant Physiol. **20** 475-94 (1969).

Sikorowska, Celina: Selenium content in the water supply in Poland. Rocz. Panstw. Zakl. Hig. **16** 11-16 (1965) (abstr. in C.A. **62** 15904B (1965)).

Slater, C. S., Holmes, R. S. and Byers, H. G.: Trace elements in the soils from the erosion experiment stations, with supplementary data on other soils. U. S. Dep. Agr. Tech. Bull. **552** 1-23 (1937).

Spåre, C–G. and Virtanen, A. I.: On the occurrence of free selenium-containing amino acids in onion (Allium cepa). Acta Chem. Scand. **18** 280-82 (1964).

Steenbjerg, F.: Planternes ernæring (DSR Forlag, Den kgl. Veterinær- og Landbohøjskole, København, 1965) 895 pp.

Steinness, E.: Determination of traces of selenium in biological tissue by neutron activation. Int. J. Appl. Rad. Isotop. **18** 731-34 (1967).

Stoklasa, J.: Über die Einwirkung des Selens auf den Bau- und Betriebsstoffwechsel der Pflanze bei Anwesenheit der Radioaktivität der Luft und des Bodens. Biochem. Z. **130** 604-43 (1922).

Swaine, D. J.: The trace-element content of soils. Commonw. Bur. Soil Sci., Tech. Comm. **48** (Rothamsted Exp. Sta., Harpenden, England, 1955) 157 pp.

Tanhuanpää, E.: The influence of dietary methyl linoleate on swine tissue lipids with special reference to nutritional muscular and liver dystrophy. Acta. Vet. Scand. **6** suppl. 3 (1965) 68 pp.

Tappel, A. L. and Caldwell, K. A.: Redox properties of selenium compounds related to biochemical function. 1st International Symposium on Selenium in Biomedicine, Oregon State University, 1966. (Edited by O. H. Muth) (AVI Publ. Co., Westport, Conn., 1967) 345-61.

Taussky, H. H., Washington, A., Zubillaga, E., and Milhorat, A. T.: Determination of trace selenium in biological fluids and tissues. Microchem. J. **10** 470-84 (1966).

Tomlinson, R. H. and Dickson, R. C.: Determination of selenium in human tissues by means of activation analysis. Proceedings: 1965 International conference: Modern Trends in Activation Analysis, College Station, Texas, 19-22 April 1965. Edited by John P. Guinn. (College Station, Texas, 1965) 66-70.

Trelease, S. F. and Trelease, Helen M.: Selenium as a stimulating and possibly essential element for certain plants. Science 87 70-71 (1938).

Trelease, S. F., Greenfield, S. S. and DiSomma, A. A.: Absorption of selenium by corn from Astragalus extracts and solutions containing proteins. Science 96 234-35 (1942).

Trelease, S. F. and Greenfield, S. S.: Influence of plant extracts, proteins and amino acids on the accumulation of selenium in plants. Am. J. Bot. 31 630-38 (1944).

Trelease, S. F.: Selenium in soils, plants and animals. Soil Sci. 60 125-31 (1945).

Trelease, S. F. and Beath, O. A.: Selenium: its geological occurrence and its biological effects in relation to botany, chemistry, agriculture, nutrition and medicine. (The Champlain Printers, Burlington, Vermont, 1949) 292 pp.

Trelease, S. F., DiSomma, A. A. and Jacobs, A. L.: Seleno-amino acid found in Astragalus bisulcatus. Science 132 618 (1960).

Turekian, K. K. and Wedepohl, K. H.: Distribution of the elements in some major units of the earth's crust. Bull. Geol. Soc. Am. 72 175-92 (1961).

Virupaksha, T. K. and Shrift, A.: Biochemical differences between selenium accumulator and non-accumulator Astragalus species. Biochim. Biophys. Acta 107 69-80 (1965).

Watkinson, J. H.: Fluorometric determination of traces of selenium. Anal. Chem. 32 981-83 (1960).

Watkinson, J. H.: Soil selenium and animal health. Trans. Jt. Meet. Comm. IV and V, Int. Soc. Soil Sci. Palmerston North, New Zealand, 13-22 November, 1962. Edited by G. J. Neale (Lower Hutt, N. Z., Soil Bureau, P. B., 1963) 149-54.

Watkinson, J. H.: Fluorometric determination of selenium in biological material with 2,3-diaminonaphthalene. Anal. Chem. 38 92-97 (1966).

Watkinson, J. H. and Davies, E. B.: Uptake of native and applied selenium by pasture species. III. Uptake of selenium from various carriers. N. Z. J. Agr. Res. 10 116-21 (1967).

Wells, N.: Selenium content of some minerals and fertilizers. N. Z. J. Sci. 9 409-15 (1966).

Wells, N.: Selenium in horizons of soil profiles. N. Z. J. Sci. 10 142-79 (1967).

Wester, P. O.: Concentration of 24 trace elements in human heart tissue determined by neutron activation analysis. Scand. J. Clin. Lab. Invest. **17** 357-70 (1965a).

Wester, P. O.: Concentration of 17 elements in subcellular fractions of beef heart tissue determined by neutron activation analysis. Biochem Biophys. Acta **109** 268-83 (1965b).

Williams, K. T. and Byers, H. G.: Selenium compounds in soils. Ind. Eng. Chem. **28** 912-14 (1936).

Williams, K. T., Lakin, H. W. and Byers, H. G.: Selenium occurrence in certain soils in the United States, with a discussion of related topics. U. S. Dep. Agr. Tech. Bull. **702** (1940), 59 pp.

Wilson, L. G. and Bandurski, R. S.: Enzymatic reactions involving sulfate, sulfite, selenate, and molybdate. J. Biol. Chem. **233** 975-81 (1958).

Wolf, E., Kollonitsch, V. and Kline, C. H.: A survey of selenium treatment in livestock production. J. Agr. Food Chem. **11** 355-60 (1963).

Wright, P. L. and Bell, M. C.: Selenium-75 metabolism in the gestating ewe and fetal lamb: Effects of dietary α-tocopherol and selenium. J. Nutr. **84** 49-57 (1964).

Wright, P. L. and Bell, M. C.: Comparative metabolism of selenium and tellurium in sheep and swine. Am. J. Physiol. **211** 6-10 (1966).

DANSK RESUME

Siden 1957 har der været en stigende interesse for selens betydning i husdyrernæringen og også en stigende interesse for veterinær-medicinske anvendelser. I dag betragtes selen normalt som et nødvendigt mikronæringsstof for højere dyr. Afdelingen for landbrugsforsøg på AEK's forsøgsanlæg Risø har derfor påbegyndt undersøgelse af selenindholdet i danske landbrugsafgrøder og efterfulgt denne med undersøgelser i relation til gødskning med selen.

Det første kapitel i nærværende rapport giver en oversigt over litteraturen om selen i tilknytning til landbruget.

Kapitel 2 behandler forsøgs- og analysemetoder. Specielt diskuteres problemer i forbindelse med at undgå tab under prøvebehandling. Selenindholdet i markafgrøder blev bestemt ved aktiveringsanalyse, medens svovl bestemtes ved en tracer-metode. Karforsøg er udført under anvendelse af radioaktivt selen (eller svovl), og målemetoderne hertil omtales. Endelig er metoder til bestemmelse af oxidationstrinnet af selen i extrakter blevet undersøgt.

Selenbestemmelser i landbrugsafgrøder fra forskellige egne af Danmark viser (kapitel 3), at selenkoncentrationen oftest er marginal. Det ønskelige indhold er 0.1-0.3 ppm i tørstoffet. I græsmarksafgrøder varierer den omkring den nedre grænse for det ønskelige indhold. Rodfrugter og korn indeholder normalt mindre selen end 0.1 ppm. Kun i få afgrøder, først og fremmest korsblomstrede, er koncentrationen tilstrækkelig høj. Man kunne derfor vente, at symptomer på selenmangel hos husdyrene skulle være hyppige. At dette tilsyneladende ikke er tilfældet, kan skyldes, at der almindeligvis gives forskellige tilskud til det hjemmeavlede foder, og at disse tilskud ofte har et væsentligt højere indhold af selen. Imidlertid synes en forhøjelse af selenkoncentrationen i det danske foder ønskelig, og en mulig vej hertil er anvendelse af handelsgødning tilsat et passende selensalt.

En række forsøg vedrører planters optagelse af selen tilført jorden (kapitel 4). Koncentrationen i planterne afhænger hovedsageligt af planteart og af det tilførte selens iltningstrin, og selvfølgelig af den tilførte mængde. Derimod er selenforbindelsens opløselighed, plantens udviklingstrin og jordtypen (forudsat det er en mineraljord) samt jordens gødningstilstand alle faktorer af langt mindre betydning. Med en given selentilførsel (som selenat eller selenit) vil man opnå en koncentration i planterne, som afhængigt af arten varierer inden for en faktor 10 (koncentrationen voksende fra kornarterne til korsblomstrede) og afhængigt af jordtypen inden for en faktor 2 (koncentrationen voksende med aftagende lerindhold). For danske mineraljorder skulle det alt i alt være muligt ud fra et begrænset antal markforsøg at forudsige virkningen af en selentilførsel inden for en faktor 3, hvilket forekommer tilstrækkeligt for anvendelse af selen i praksis.

Det er ikke indlysende, hvilken selenforbindelse, man bør foretrække. Optagelseskvotienten for selenater er høj og en del af det resterende selen vil kunne udvaskes med deraf følgende risiko for grundvandet. Selenit har en lille optagelseskvotient, og der er mulighed for en akkumulering i jorden ved gentagne tilførsler.

I flere forsøg er selenet i jord og planter søgt karakteriseret (kapitel 4 og 5). Enten selen er tilført som selenat eller selenit, er det dominerende oxidationstrin i jorden nogle måneder senere +4. Men også selen i oxidationstrinene +6, 0, -2 samt organisk bundet selen er til stede. I planter (radise) fandtes en større part af selenet som selenit end antaget ud fra tidligere arbejder.

Af mere teoretisk end praktisk interesse er, at svovlkoncentrationen i radiseblade vokser med stigende tilsætning af selen til jorden (kapitel 6). Koncentrationsforøgelsen er identisk med stigningen i sulphat i planten, og fænomenet formodes derfor at skyldes, at selenationen blokerer reduktionen af sulphat.

Principielt har man svar på de væsentligste problemer i forbindelse med anvendelse af selentilførsel til jorden. Yderligere laboratorieforsøg vil hovedsagelig tjene til at give flere detaljer; dog kan man stadig håbe på en virkelig løsning af det kritiske spørgsmål om en ideel selenforbindelse. Det næste skridt nu må være markforsøg i større skala. De forhåndenværende følsomme analysemetoder (aktiveringsanalyse, fluorometri) gør den nødvendige kontrol overkommelig.